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HEAT PRODUCTION AND LIMITATION OF DENSITIES IN SITOTROGA POPULATIONS.

STANLEY E. FLANDERS,
University of California Citrus Experiment Station,
Riverside, California.

When grain is placed in heaps or stored in bins it is subject to heating, either as a result of high moisture content or an insect infestation. According to Bailey (4) hygroscopic moisture in grain in excess of 13 per cent is accompanied by a marked increase in grain respiration and its resultant heat. This heat of respiration occurs only when the relative humidity of the surrounding air exceeds 70 per cent.

The production of heat by insects is a phenomenon very often observed, but which has received comparatively little consideration by entomologists and ecologists. The cause of heating when insects are present in numbers has not been clearly understood. It is generally believed that the heating of grain is sometimes started by insects, and that their development makes it possible for other agencies not so well understood to gain a foothold and carry on the destruction. Back and Cotton (2), in 1924, summarized all available information concerning it and stated that "probably the most prevalent opinion is that heating is due to fermentation started by the attraction of moisture to the faeces of the insects." Doran (8) expressed the opinion that heating of grain was due to the conversion by the insect at the time of transformation of a certain amount of so-called "latent heat" into sensible heat. That a certain amount of heat energy was expended in the operation seemed especially probable owing to the more or less complete histolysis during the pupal stage. Schwarz (Back

and Cotton) (2) stated that the accumulation in numbers of insects produced considerable heat, and referred to the hibernation of *Megilla maculata*.

An early record in America of this phenomenon of heating is a note by Howard (11) in 1888, concerning the heating of seed by *Bruchus scutellaris*. This heating he thought to be due in great part to a mechanical cause—the gnawing of the peas by the beetles and their larvae. Back and Cotton (2) believed that the developing larvae were quite as important in initiating the heating process as the adults, if not more so.

TABLE I.

Effect of the Developing Larvae of *Bruchus quadrimaculatus* on the Temperatures of Two 240-pound Sacks of Chick Peas.[1]

(Three hundred seeds, approximately six ounces, were examined from each sack.)

Sack	Total Number of Insects	Developing Larvae	Full-grown Larvae, Pupae, and Adults in Seeds	Temperature of Seeds
A	1,097	167	930	89° F.
B	1,271	695	576	102° F.

Seeds from sack B had 528 more developing larvae, and 354 fewer full-grown larvae, pupae, and adults in seeds than those from sack A.

They also found "that fumigation will kill such heat and permit the grain to return to normal temperature," and conclude, "unless it can be proved that bacteria, molds, or other agencies causing heat are also killed by HCN or carbon disulphide . . . the activity of certain stored product pests is directly responsible for the development of heat in grain in certain instances. . . ."

Possibly the earliest recorded observation pertaining to the heating of grain by insects was that by Duhamel du Monceau and Tillet (9) in 1762. They commented:

> "Is it produced by the heat of the insects themselves which are found assembled in great numbers? That could be, because it is known that there is a considerable degree of heat in hives that

[1]Data given by Back and Cotton (2).

> are well populated with bees, and that grain infested with weevils or false clothes moths is sometimes very hot. We will say later that there is no sensible heat in the heaps where there are very few caterpillars and that the heat is dissipated when most of the caterpillars have changed into moths."

Then they reported the following experiment:

> "On July 26, 1761, in Angoumois, France, M. de Tapannat filled a small barrel with small heads of wheat filled with kernels. Under that condition they heated up and soon afterward moths emerged from the grain. This grain gradually lost its heat, but in September it heated again. On September 8, 61 kernels were examined and 9 showed the emergence holes of the first brood, and 29 contained the larval and early pupal stages of the second brood."

If, as seemed probable, the heat of infestation is simply an effect of the activity of the infesting insect population, could it possibly be used to measure such populations in a series of equal units of corn stocked with single generations of *Sitotroga cerealella* of different densities? To answer this question the following experiment was undertaken:

Twelve one-gallon jars were filled with common white corn, which had been previously fumigated with carbon disulphide in a vacuum fumigator. Each jar contained approximately 9,100 kernels of corn. Ten of the jars were infested with newly-hatched larvae of *Sitotroga cerealella*, and two were left uninfested. The ten infested jars were stocked as follows:

2 jars with approximately 1 larva per kernel.
2 jars with approximately 2 larvae per kernel.
2 jars with approximately 2½ larvae per kernel.
2 jars with approximately 3 larvae per kernel.
2 jars with approximately 4 larvae per kernel.

These larvae were placed on the corn over a period of two days, at a temperature of 29° C. A centigrade thermometer was kept immersed in each jar.

The jars were divided into duplicate sets of six each and were kept at varying temperatures. Each set served as a check upon the other. The heat of infestation was determined each day by subtracting the temperatures of the uninfested jars from those of the infested ones. This experiment was completed in 60 days. The individual life cycle was about 40 days.

In one set, which was kept at a temperature of 29° C., the heat of infestation was first apparent six days after the eggs hatched. The second set was removed to a temperature of 20° C., but as the heat of infestation did not become apparent over a period of 13 days, it was replaced at a temperature of 29° C. and the heat became apparent the next day. If more delicate means of temperature measurements had been used, the heat of infestation probably would have been noted earlier.

It was found that the heat of infestation increased as the larvae increased in size, until most of them had become full grown and had spun their cocoons. From the time the first moth emerged the heat decreased until emergence was complete, then the heat of infestation disappeared and the temperature returned to that of the uninfested jars. The more dense populations have the shorter life cycles, owing to the added heat they produce.

In an environmental temperature range of 20° C. to 30° C., the heat of infestation curve is largely independent of environmental temperature fluctuations, particularly during the later stages of development. In small units, as in the experimental sets, a temperature of 34° C. lowers the heat of infestation somewhat and a temperature of 8.5° C. eliminates it entirely, when measured by ordinary thermometers.

The total heat of infestation for each jar, as indicated by the sum of the daily readings, was greatest in the jars stocked with 2 or 2.5 larvae per kernal, and least in those stocked with 1, 3 and 4 larvae per kernel.

In order to determine the amount of infestation which produced the total heat in each jar, a count was made of the infestation in a representative sample of 200 kernels from each. The infestations and their corresponding heats are shown in Table II.

More refined methods of temperature measurements should reveal a closer correlation than was obtained in this test.

This test indicated that the maximum possible single generation population of *Sitotroga* in a mass of corn infested only 40 per cent of the kernels and that 99 per cent of the infested kernels had only one individual per kernel out of a possible three or four.

In jars having a single generation population stocked with two or more larvae per kernel, the maximum infestation

occurs at the top. This is apparently due to the fact that the newly-hatched larvae tend to migrate downward, become crowded toward the bottom, and come in conflict with each other. They were observed to attack each other savagely and persistently. Duhamel and Tillet often observed three or four dead caterpillars on one grain, of which only one caterpillar had taken possession. On one occasion two were observed apparently fighting. After several minutes one was dead and the other was seen working its way into the kernel.

TABLE II.

Temperature Correlations Between Unequal *Sitotroga* Populations in Equal Amounts of Corn.

Approximate Number Larvae Released per Kernel	Set 1			Set 2		
	Infestation (Per Cent)	Sum of Daily Temperature Readings for 47 Days (Degrees C.)	Maximum Daily Readings (Degrees C.)	Infestation (Per cent)	Sum of Daily Temperature Readings for 39 Days (Degrees C.)	Maximum Daily Readings (Degrees C.)
1.0	22	54	2.2	18.0	30	1.3
2.0	28	75	2.8	35.0	60	3.0
2.5	40	96	3.5	31.5	48	2.1
3.0	31	69	2.5	26.0	41	1.6
4.0	28	68	2.4	22.5	33	1.4

The maximum daily heat of infestation in each jar occurred several days prior to the emergence of the first adult. The emergence of the adults was completed in about 20 days.

They were never able to find more than one caterpillar in a kernel. It seems probable that after a larva enters the kernel and becomes sluggish from feeding, it falls easy prey to another that follows later. Corn infested with mixed broods of *Sitotroga* may never have over 50 per cent of the kernels infested with living individuals of the same age at any one time. *Sitotroga* larvae, unlike those of *Plodia interpunctella*, never feed on the eggs of their own species, even when deprived of other food material.

As the initial population of larvae per grain exceeded two, the adults from 100 larvae fell off sharply at the following rates:

Set 1	Set 2	Initial Population Larvae per Grain
Adults per 100 Larvae	Adults per 100 Larvae	
22	18.0	1.0
14	17.5	2.0
16	12.6	2.5
10	8.7	3.0
7	5.6	4.0

Maclagan (14) obtained a similar effect in his experiment upon the interspecific effect of mixed populations of *Sitotroga cerealella* and *Calandra granaria* in 1,000 grains of wheat, on the reproductive rate of the moth, as follows:

Progeny per Moth[2]	Initial Population of Moths	Weevils
18.0	10	None
11.6	20	10
8.7	40 (½ females)	10

Maclagan draws the conclusion "that the addition of weevils to the environment brings about a profound reduction in the moth population." Maclagan believed that the decrease in progeny per moth was due to a decrease in reproductive rate of the parents.

SUMMARY.

The two population phenomena of ***density limitation*** and ***heat production*** considered in this paper, appear to be mechanical in nature.

In equal environments the number of survivors per unit of high initial populations varied inversely with the initial population as a direct result of internecine activity.

[2]According to Back (1) each female moth deposits about 150 eggs.

The amount of energy transformed by the developing larvae into heat increased until the larvae ceased feeding. This heat can be used as a measure of the relative abundance of larvae.

The number of larvae of the same age living at the same time in a given amount of corn rarely, if ever, exceeds in number 50 per cent of the kernels.

It appears possible that these phenomena are common to all insect populations which subsist upon the media in which they live.

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NOTES ON HABROCYTUS CEREALELLAE, PARASITE OF THE ANGOUMOIS GRAIN MOTH.[1]

B. B. FULTON,
North Carolina State College,
Raleigh, N. C.

During the early part of 1932 the writer became interested in studying the life history and habits of a Chalcoid parasite of the Angoumois grain moth, *Sitotroga cerealella* (Olivier). The species was identified by C. F. W. Muesebeck as *Habrocytus cerealellae* (Ashmead). Later in the year an excellent study of the same species was published by Norman S. Noble (1). After reading over Noble's work, the writer decided to prepare the present paper giving briefly a part of the life history work and devoting the remainder to a discussion of certain habits of the adult. The remarkable process of oviposition seems to be specially worthy of greater emphasis. The habits of both host and parasite make them ideal material for the study of all the activities associated with oviposition.

METHODS.

The technique used for rearing the parasites under observation was different from that employed by Noble, who succeeded in getting oviposition on host larvae removed from the grain and confined with the female parasites in small tubes. The writer made a few unsuccessful attempts to induce the parasites to oviposit on free moving larvae and then adopted the scheme of confining the host larvae in small glass cells made in the following manner. A glass tube just large enough to hold a full grown Angoumois larva was cut into sections about one centimeter long. One end of the tube was touched on a smear of glue and then pressed against a piece of tissue paper. After drying, the edges of the paper were trimmed off, leaving a circular cap over the end of the tube. Host larvae were pushed into the other end of the tube, head first, and held there by a small cotton plug. Several such cells were placed in holes in a card so that they would stand with the paper cap upper-

[1]Published with the approval of the Director of Research as Paper No. 66 of the journal series.

most, and the whole card was then pushed under a large inverted Petri dish used for confining the adult parasites. A cotton wad saturated with sugar water was kept under each dish. The female *Habrocytus* would climb to the top of such a cell and oviposit through the tissue paper cap the same as through the seed coat of an infested grain. If the cell was placed close to the side of a glass dish the whole process of oviposition could be watched with a binocular microscope. The number of eggs in each cell could be easily counted through the glass and further observation made on the development of the parasite without disturbing it.

LIFE HISTORY.

The life cycle of *Habrocytus cerealellae* was run at two constant temperatures, 25° C. and 30° C. Eggs were deposited in the laboratory on host larvae left in the cages for periods of from four to seven hours. They were then kept under constant temperature conditions except for short periods while records were taken. The table below gives the time elapsed between placing the eggs under constant temperature and the appearance of adults.

	25° C.	30° C.
Average of 33 males	12.7 days	.....
Average of 16 males	.....	10.2 days
Shortest period, males	11 days	10 days
Longest period, males	14 days	11 days
Average of 57 females	13.7 days	.....
Average of 26 females	.....	11.1 days
Shortest period, females	12 days	10 days
Longest period, females	15 days	13 days

At 25° C., the egg incubation period was over one day and less than two days, probably about a day and a half. The active larval stage was four or five days and the prepupa about one day, but in one case was known to be less than 20 hours. The pupal stage was five or six days for males and six or sometimes seven days for females. At 30° C., the incubation period was about one day; in one case certainly not over 23½ hours and in another case not less than 23 hours. The active larval period was 4 or 4½ days and the prepupa about one

day or a little less. The pupal stage was four or sometimes five days for males and five or sometimes six days for females.

At the beginning of the prepupal period the larva stopped feeding and a considerable amount of greyish fecal matter was passed out before any change in form was noticeable. At both temperatures the pupa was yellowish in color for a little more than half the total pupal period and black for the remainder.

The molting of the pupal skin was observed several times. A transverse break occurs at the neck region separating the head portion while the thoracic covering splits longitudinally. By bending the body the thorax is freed first, followed by the legs and wings. The head remains covered until the front legs are freed and used to push off the head covering. This comes off in one piece except for the funicles of the antennae which remain covered until sometime later when they are cleaned off with the front legs.

The adult parasites remained in the cells for a period generally less than one day, but in a few cases longer than one day. Cells closed with the seed coat of corn apparently imposed no greater obstacle to freedom than those covered with tissue paper. Exit was accomplished by cutting a small round hole in the cell cover. The exit holes of the parasite in grain differ from those of the host by being of much smaller size.

Five females and three males were confined individually or paired from the time of emergence until they died. Others escaped before completing the life span. All were fed on sugar water. The three males lived for 19, 25 and 30 days. The length of life and number of eggs deposited by each female are as follows: 27 days, 97 eggs; 45 days, 240 eggs; 47 days, 182 eggs, 51 days, 284 eggs; 78 days, 676 eggs. The last four periods mentioned include seven days confinement in a refrigerator.

The preoviposition period was usually one or two days, after the adult had left the grain, or two to three days after reaching the adult stage. The most prolific female did not oviposit until the sixth day after leaving the grain.

In this species as in many other Hymenoptera, the automatic regulation of sex insures maximum reproductive capacity. Fertilized females produce a large excess of females, but unfertilized females produce only males. As long as there are enough

males to mate with all the females productivity is maintained at a high level by the excess of female progeny. At any time there is a scarcity of males more will be produced by the reproduction of unfertilized females. The proportions of the sexes in the progeny reared from five mated females were as follows: males 5, females 8; males 10, females 37; males 0, females 38; males 3, females 37; males 7, females 19; total males 25, females 139. The proportion of males would probably have been higher if the rearing of progeny had not been discontinued before all the eggs were laid, for there seemed to be a tendency for an increase in male offspring several days after the male parent had died. The proportion of males in wild stock emerging from three ears of corn was higher, being 35 males to 43 females.

HOST RELATIONS.

The time required for a complete life cycle of the Angoumois moth at a favorable temperature is a little over a month. Considering the short life cycle of *Habrocytus*, the number of eggs laid and the fact that a large proportion of the offspring are females, the reproductive capacity of the parasite must be close to three times as great as that of the host. Two other factors handicap the parasite and decrease its ability to completely parasitize a host culture. These are (1) the limited portion of the host cycle during which oviposition by the parasite is possible and (2) the fact that the female parasite does not discriminate between active host larvae and those that have been previously paralyzed. Habrocytus does not deposit eggs in grain containing Angoumois pupae and cannot reach the host larva until its cavity in the grain has extended to the seed coat, but in many cases the host larva is parasitized before the circular exit hole is completed.

Several parasite eggs were sometimes found in one grain. In supplying host material for breeding purposes it was found that previously paralyzed larvae in good condition were as acceptable for oviposition as active larvae. In the breeding containers, when a limited number of host larvae were made available, as many as 17 eggs were deposited with a single host. As a result of this lack of discrimination on the part of the parent several offspring may hatch on one host, but usually after about a day only one will remain. Since many of

them hatch on the walls of the cell, they have to wander about before finding the host and when two of them meet, one is destroyed. The one that happens to meet the flank of the other will take hold at once with its minute sickle-shaped jaws and the struggle is soon over. Unhatched eggs also fall prey to the wandering larvae. One was observed to feed on three eggs, leaving them in partly collapsed condition. In another instance a larva was hatching from an egg and as soon as it poked its head through the chorion it came into contact with another egg. Without stopping to free the remainder of the body from its own egg, the larva fastened its jaws into the one before it and drew out part of the contents, leaving a hollow place in the side. In one cell, three partly grown larvae were found one day after hatching, two were present on the second day and only one on the third. In three cells, two parasites continued to develop to the pupa on the same host. In one of these two adult males appeared, but both were dead in the cell the next day. In another two females had a similar fate and in the third, two females matured and one emerged from the cell, leaving the other dead.

A few Mediterranean flour moth larvae placed in glass cells were not parasitized. A cell containing a rice weevil larva and two others with Angoumois larvae were placed in a cage containing one female parasite. The first cell received two eggs and the others one and three. A female parasite was reared on the rice weevil larva in average time.

OVIPOSITION.

When the eggs of *Habrocytus cerealellae* were first found, their presence in a grain of corn with no apparent means of entrance was a mystery.

Close examination of the seed coats covering Angoumois cavities revealed one or more minute holes when parasites were present. These holes were only .04 mm. in diameter, while the Habrocytus eggs were .16 mm. in diameter by .55 mm. to .6 mm. in length. The first supposition was that the eggs must swell after being deposited, but fully developed eggs dissected from females were as large as those deposited. The finding of an egg projecting part way through a hole then led to a theory that the egg must be forced through the hole a little at a time. To determine this some infested corn grains were carefully

cut away on the side leaving the exit cover intact and a piece of glass cover slip was glued over the exposed side of the cavity. These grains were placed upright in pieces of clay near the side of a glass container containing female Habrocytus. A binocular microscope was then fixed in a horizontal position so as to obtain a clear view of the interior of the cavity and the space above. In this way the whole process of oviposition under normal conditions was observed a number of times.

The female goes over the place above the host larva with antennae pointed downward and in constant motion. Finally

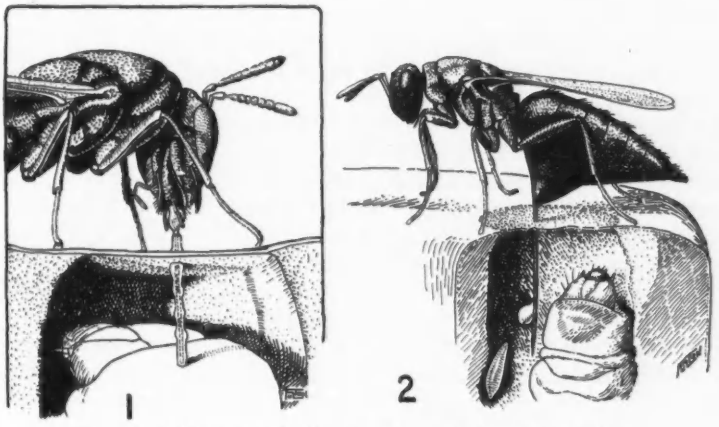

FIG. 1. Habrocytus feeding at tube made by her ovipositor.
FIG. 2. Oviposition.

selecting a suitable spot she elevates the back, bringing the tip of the abdomen against the seed coat cover. She taps about over the surface and at some point the ovipositor comes out of its resting place on the ventral median line of the abdomen and the abdomen is again directed backward, the ovipositor assuming a vertical position from the base of the abdomen. Several large sternal plates which cover the proximal portion of the ovipositor are stretched forward (Fig. 2).

Drilling proceeds by a slow twisting movement of the stylet sheath and a rapid up and down movement of the stylets, while the insect pulls downward with the legs. On breaking through the seed coat the ovipositor is quickly extended and

makes several rapier-like thrusts into various parts of the host cavity and then is almost withdrawn while the parasite rests for a short time. If the larva is not stung the first time the performance is repeated until it is. The tip of the ovipositor pierces the skin of the host larva and is very quickly pulled back out of the way, while the larva writhes for several seconds before becoming quiescent.

The ovipositor is extended again as far as possible into the cavity and bending rather sharply in different directions just inside of the cavity is used as a probe to explore the space within. The stylets are frequently extended as far as possible beyond the sheath to reach the more distant walls of the cell. After probing in all directions, the stylets are withdrawn so that they extend only a little beyond the sheath. With the ovipositor in a vertical position the insect stands quietly for a while. Then it quivers slightly and an egg suddenly appears, swelling out like an inflated rubber balloon, on the ventral or anterior side near the tip of the stylets (Fig. 2). The ovipositor is then bent and the stylets extended until the egg touches the host or the side of the cavity where it sticks and the ovipositor is withdrawn and assumes its resting position. The tip of the abdomen is usually brought forward again and tapped about until it touches the hole made by the ovipositor and a small droplet of liquid is left on it. The whole process may be completed in a few minutes. The egg passes through the shaft of the ovipositor without causing any appreciable enlargement of the organ, which was clearly visible under magnification during the observations. The hole through the seed coat is no larger than the external diameter of the ovipositor which measures .04 mm. The diameter of the space within the ovipositor, measured from cross-sections, is only .011 mm. The greatest diameter of the egg is .16 mm. and the mean diameter about .12 mm. Even allowing for a slight expansion of the parts during oviposition, the egg would have to be compressed to about one-twelfth of its greatest diameter. The cross-section area of the widest part of the egg is 144 times as great as the cross-section area of the space it comes through. By making a wax model of an egg enlarged 200 diameters, the approximate volume was measured by displacement. From this it was computed that if the entire egg were reduced in diameter only to the size of the ovipositor itself, it would be

5.17 mm. long. If reduced to the internal diameter it would be 46 mm. long. But the ovipositor of even a large specimen measures only 1.8 mm. The only logical conclusion is that the egg goes through the ovipositor like sand through an hour glass. While one part is leaving the distal end of the ovipositor another part is still entering the proximal end. Only a small part of its bulk is contained in the ovipositor at one time.

Several questions naturally arise concerning this remarkable manner of depositing eggs. Does the insect profit in any way by having an egg much too large for its ovipositor? Do any other species present a similar phenomenon? How can the egg stand the strain that it must be subjected to during the process? What is the mechanism by which it is accomplished?

In regard to the first question, it seems reasonable to suppose that a larger hole in the grain might permit the entrance of mites or other predators to feed on the parasite or host. A thick ovipositor would also be more destructive to the host when it is stung. Two advantages in a large egg are suggested, a quicker start in life to shorten the life cycle, and a greater ability in feeding on the host by a large newly hatched larva.

In looking over literature on other species of Chalcid flies, no descriptions were found which gave the thickness of the ovipositor. A number were found which gave both the length of the female and the dimensions of the egg. After comparing these figures with the dimensions of the female *Habrocytus* and its egg it seems probable that egg stretching is not an uncommon process among these insects. *Habrocytus* does not appear to have an unusually small ovipositor when compared with figures of other Chalcid species in the act of ovipositing. The size of the egg of *Monodontomerus aereus* as given by Muesebeck (2) is .6 to .65 by .2 mm., considerably larger than the *Habrocytus* egg. The females of the two species are of about the same length, but the former is considerably more slender as shown by the cut. If the cut is assumed to be a medium-sized female and the egg drawn on it to the same scale from the dimensions given, its greatest diameter would be about one-third the width of the abdomen. The only reference that the writer has yet found, which even suggests that Chalcid eggs may be too big for their ovipositors is the recent work by Noble (1) on the present species. He gives the dimensions of both ovipositor and eggs and states "that they

must undergo considerable compression during their passage down the ovipositor tube."

The elastic qualities of the thin membrane which constitutes the shell of the egg can be demonstrated in several ways, but no method tried closely approached the conditions of oviposition. A small glass tube was drawn out at one end and filled with water. A mature egg from the ovary was dropped in the large end and allowed to sink into the small end. The large end was then plugged with clay, excluding air bubbles. By pressing on the clay plug the egg could be forced farther into the capillary end. In this way it is possible to constrict an egg to about three times its normal length. If the tube is made smaller the egg will stick and can not be forced through. If an egg is placed in water on a slide and pulled lengthwise by two blunt needles, the portion between will stretch out and decrease in diameter somewhat like the drawing out of a heated glass tube. The needles cut and tear the chorion before it breaks in the middle. If a cover glass is placed over the egg and pressed down, the egg will flatten out to several times its original area before it breaks. If the pressure is released before it breaks it will resume its original shape. By the last method the chorion is stretched not only transversely but to some extent lengthwise. In passing through the ovipositor it would be stretched lengthwise only. The part passing through the ovipositor must be actually drawn out to a tube as small as the passage, for there is not room enough to permit any folding of the chorion. The inside area of the portion of the ovipositor through which the egg passes is equal to the area of a middle section of the egg one-fifth of its length. Such a distortion of the egg chorion is the most difficult part of the picture. The entire contents of the egg could be crowded into two-fifths of its length by a tangential stretching that would no more than double the area of that part. The viscous contents of the egg could flow from the portion in the body to the portion outside, through the stretched out portion in the ovipositor. It is a fact that when the egg first issues from the ovipositor the end that comes out first is larger and the other end is partly retained in the tube until the egg is placed. Within about a second, however, a shifting of the contents takes place and the greatest diameter is found to be near the end that came out last.

MECHANICS OF OVIPOSITION.[2]

The process of oviposition as observed in this species seemed so much like a conjurer's trick that an examination of the anatomy of the ovipositor was necessary to explain it. The writer had assumed at first that the egg was forced into the ovipositor by pressure from within the body. Cross sections of the external part of the ovipositor showed the stylets locked to the stylet sheath by a tongue and groove arrangement, but the two portions of the sheath and the two stylets were not held together by any mechanism that would stand any great amount of internal pressure. Within the body no muscular structures were found for forcing the egg into the ovipositor. The mechanism seemed to be designed to pull the egg into the ovipositor by the action of the stylets. This explains also why the surface of the egg is dotted with minute studs or knobs, a feature which at first seemed an unnecessary waste of space. When at rest, the shaft or external part of the ovipositor is enclosed between a pair of narrow plates extending the entire length of the abdomen and which are connected on the median line by conjunctiva (Fig. 13, r). Paralleling these inner ovipositor plates is a pair of broader, outer ovipositor plates (Figs. 17, 19, s). The cephalic portions of both pairs are covered by the sternal plates, which during oviposition are pulled forward and wrap tightly around the base of the ovipositor (Fig. 19, y). At the base of the shaft the stylet sheath is enlarged and articulated on the cephalic ends of the inner ovipositor plates. The tongued and grooved portion bearing the stylets is continued on the cephalic side into two narrow arms that spread apart in the abdomen and describe semicircular courses in a caudal direction (Figs. 13, 17, g). They are supported by thin fan-shaped extensions of the lateral margins of the inner ovipositor plates (t). The stylets continue around the sheath arms and are held to them all the way by a tongue and groove structure (Figs. 16, 17). The ends of the stylets are attached to fulcral plates (q) lying outside of the fan-shaped supports. The fulcral plates articulate both

[2]In a recent paper by Snodgrass, R. E., Smithsonian Misc. Collections, v. 89, No. 8, 1933, the parts of the ovipositor are homologized under terms differing from those used in this paper. The synonymy is as follows: stylets=1st valvulae; sheath=2nd valvulae; fulcral plate=1st valvifer; inner ovipositor plate=2nd valvifer; outer ovipositor plate=9th tergum.

on the inner and outer ovipositor plates so that when the position of these plates is shifted by the muscles connecting them the fulcral plates describe a semicircle and work the stylets in and out. No attempt was made to work out the complicated system of muscles associated with the ovipositor.

The ovaries consist of three egg tubes each (Fig. 15, n). The oviducts (o) empty into a vagina which is flattened horizontally and extends to and includes the sheath arms. On each side the ventral vaginal wall (j) attaches to the fan-shaped support just beneath the arm, but the dorsal wall (i) continues over the sheath arm as an almost invisible sheet and attaches to the caudal border of the fan-shaped support and anteriorly to the conjunctiva connected with the sternal plates. The fulcral plate lies outside of this lateral extension of the vaginal wall, but is attached to it along a part of the cephalic edge of the plate. When the fulcral plate moves it carries this thin wall with it. The caudal portion forms a pocket over the caudal end of the sheath arm and falls into loose folds when the stylet is pulled in. The vaginal cavity includes the stylets, sheath arms and the outer faces of their fan-shaped supports. The muscle-bearing parts, including the fulcral plates and inner or dorsal faces of the fan-shaped supports, are in the body cavity. The outer side of each sheath arm bears a row of minute spines (Figs. 16, 17), which may help to keep the stylet in position, but this hardly seems necessary because of the tongue and groove structure. Attached to the base of each stylet and to the dorsal vaginal wall is a peculiar spindle-shaped structure (Fig. 17, u). It is formed of colorless cuticula, appears to be hollow and bears deep transverse ridges or corrugations. It might be a lubricating organ or it might serve to lock into the minute spines of the sheath arm to hold the stylet in a fixed position. Opening into the dorsal wall of the vagina cephalad to the oviducts is a pair of bean-shaped pouches (Figs. 15, 19, p) filled with fine granular matter. The cephalic side of each pouch bears a smaller pouch which in freshly killed specimens is white in color. The cephalic portion of the dorsal wall of the vaginal cavity is supported by a Y-shaped structure (Figs. 13, 15, 19, h), the arms of which lie directly over the sheath arms. Some strands of muscle are attached externally to each arm of the "Y" and probably attach laterally to the sternal plates. They would serve to pull the wall tightly against the base of the

ovipositor. The median portion of the dorsal vaginal wall is raised to form a passage for the eggs.

The ventral wall of the vagina is supported by a large turgid sac (Figs. 15, 19, x) which empties into it near the base of the ovipositor shaft. An elongated gland with a small central canal empties into the sac on the ventral side. Another elongated gland with a large central cavity empties by a narrow tube directly above the base of the shaft, and a tongue-like structure extends from this point a short distance into the ovipositor. These two glands (Fig. 19, w) correspond respectively to the acid and alkaline glands of the honey bee, both in appearance and position. The material in the large sac may have another function. It is a clear viscous liquid that hardens when exposed to air, and has the same appearance as the secretion used to form feeding tubes as will be described later. The quantity of it also suggests this function.

Below the openings of the oviducts, is a small spermatheca (Figs. 14, 19) consisting of a clear walled sac containing a smaller opaque yellowish sack emptying through a winding tube of the same appearance. On one side of the outer sac there appears to be a gland emptying into the tube. A nerve entering the abdomen dorsad to the vagina terminates in a large ganglion (Fig. 19, v) which is attached by nerve branches to the wall between the two oviducts. Within the vaginal cavity both walls are covered with very minute spines (Fig. 13, i, j) directed toward the shaft of the ovipositor. The mesal surfaces of the stylets bear minute spines and curved ridges (Fig. 16) directed toward the distal end. The exact nature of these structures is difficult to determine, for they are without color and extremely minute. They seem to be borne on a thin flange that is folded back on the main part of the stylet. From the proximal end of the stylet they increase in number and prominence toward the point where the stylets, in normal position, enter the shaft of the ovipositor. Beyond this point they fade out, but the edge of the flange seems to be serrate for nearly its whole length. By transmitted light, correctly adjusted, the entire ovipositor shaft shows regular dark transverse bands which may be caused by minute ridges.

With the foregoing morphological background the writer's theory of the method of oviposition can now be described. The egg comes through the channel in the dorsal wall of the

vagina small end first. As it passes into the narrow angle between the sheath arms the small end is further constricted by pressure from all sides, the large sac pressing on the caudal side, the stylets laterally and the dorsal vaginal wall backed by the sternal plates on the cephalic side. It is prevented from moving back by the spines on both walls of the vagina engaging the knobs on the egg's surface. The stylets now moving downward pull the end into the ovipositor. By stretching the chorion nearly devoid of contents through the ovipositor there would be little lateral pressure exerted. As soon as a part has reached the distal end the contents of the egg could start flowing through and expanding in the free end below. The movement of the stylets would keep the chorion passing through the ovipositor in stretched condition until the whole egg had passed through.

Although the whole process seems utterly impossible, the bare fact remains that the egg actually goes through the ovipositor without causing noticeable expansion. The most difficult feature to account for is the way in which the first part of the chorion to enter the ovipositor is prevented from snapping back when the stylets move up for the next downward stroke. No devise could be found for preventing the recoil, but it may be that the stylets are jerked back so quickly that the recoil does not have time to take place. In order to find out whether further observations would substantiate the theory, an observation cell as previously described was set up and a light was focused on it so that the 48× objective of the binocular microscope could be used. By this means every detail of the movements of the ovipositor could be observed. Before the egg appears the stylets, working together, start moving up and down very rapidly. In about a second the end of the egg protrudes from between the edges of the stylets near the tip, but sometimes nearer the middle. The pumping action of the stylets continues and the egg appears to grow by pulsations corresponding to the downward thrusts of the stylets.

Further observations on the use of the ovipositor as a probe showed that two movements are involved, a simple bending forward or back, and a rotation of the ovipositor up to at least 90° when reaching in any other direction. Bending does not interfere with the movement of the stylets, so it must be

accomplished by the sheath alone. At the tip, the sheath is a single piece, but above it separates into two halves, one of which overlaps the other. A pull on one-half with a push on the other could therefore cause the organ to bend. But the two halves are also joined at the base. An examination of that part shows that the two sides are held together by a bridge (Figs. 6, 13, k) made up of thin transverse plates. By manipulating the two sides of the base with two needles, the ovipositor can be made to bend for the laminated bridge permits a slight shifting of the parts. Transversely the bridge is quite rigid so that the whole base rotates as one piece.

While probing, the ovipositor seems to be sensitive to tactile stimulation. A number of minute setae (Figs. 4, 5, c) on both the sheath and stylets probably serve this function. Minute oblique pores (b) can be seen leading from the central canal (a) of the stylets to the outside and similar ones passing through the sheath. A central canal in the sheath is apparent only near the tip where the two halves are united. Snodgrass (3) describes similar pores in the honey bee sting and claims that they are sensory pores and that the central canals of both sheath and stylets connect with the body cavity. Pemberton and Willard (4) describe pores in the sheath of an Ichneumonid and claim that they are for the injection of poison. If the ovipositor has any sensory function, some connection with the body cavity would appear to be necessary for the existence of nerves. In the present species no connection of the central canals of the stylets with the body cavity could be made out. If any closed cavity exists in the upper part of the sheath it could not be discovered in any of the cross section preparations, but the cavity between the sheath and stylets is tightly enclosed and definitely terminates in the canal at the tip of the sheath.

FORMATION OF FEEDING TUBES.

One cannot help admiring the efficiency of the diminutive Chalcid ovipositor. In addition to its primary function as a passage for eggs, it serves as a drill, a sting, a probe, and as an organ for obtaining food. The last use of the ovipositor by Braconids and Chalcids has been discussed by other authors. With the apparatus used for observing the oviposition of the present species the formation of feeding tubes could be observed in such detail that a description of it seems worth while. When

preparing to feed, the actions of the parasite are entirely different from its behavior preceding oviposition. After stinging the host larva there is no probing of the cell, but the ovipositor is thrust deeply into the first segment of the larva and remains there for a short time while it is worked about. In the first case observed the head of the larva was not very close to the top of the cell and the parasite made five attempts to alter its position by pulling it up with the ovipositor in some way hooked in the flesh. Each time the ovipositor was withdrawn the larva fell back and the parasite finally formed a feeding tube which was apparently too long, for it broke while the ovipositor was being removed. After watching a second unsuccessful attempt the writer changed the position of the larva in the cell and then observed the formation of several feeding tubes.

After probing into the body of the host the ovipositor is withdrawn so that only the tip extends through the hole in the seed coat. A clear viscous liquid begins to ooze from all parts of the ovipositor, but more copiously near the tip. At first appearing in nodules the material is spread by a twisting and up and down movement of the ovipositor. Some of the material accumulates on both sides of the hole in the seed coat. By working the ovipositor gradually downward for two or three minutes a continuous coating is built up until the ovipositor reaches the host. Then it is slowly moved about and apparently the original puncture is located. The tip again enters the host with little effort and is slowly forced in as far as it will reach. It remains there about two minutes with only a slight twisting movement. Then the parasite begins withdrawing the ovipositor very slowly and carefully. During this movement the ovipositor makes slight but abrupt twists and the stylets keep working up and down alternately. These movements permit the gradual withdrawal of the ovipositor without placing a great strain on the tube. Within two or three minutes the ovipositor is entirely out, leaving a small extension of the tube above the seed.

The parasite places the ligula of the labium over the end of the tube and draws the body fluid of the host through it (Fig. 1). The ligula is a single fleshy colorless lobe having several transverse bristle-bearing ridges on the ventrocephalic and lateral faces, and having a small tube within, opening at the

tip. Feeding continued for fifteen minutes to one hour. Immediately or soon after feeding ceased the tube was broken by the parasite thrusting its ovipositor through it. In some cases an egg was deposited at the same time, but in others the action seemed to be only for the purpose of destroying the tube.

SUMMARY.

The life cycle from egg to adult at 25° C. averaged 12.7 days for males and 13.7 days for females. At 30° C. it averaged 10.2 days for males and 11.1 days for females. Eggs obtained during the life of a single female varied from 97 to 676.

Parasitism is possible only during the larval stage of the host after its cavity reaches the seed coat. Parasites oviposit on both active and paralyzed larvae. Parasite larvae are cannibalistic and rarely will more than one mature on the same host. One was reared on a rice weevil larva.

The greatest diameter of the egg of *Habrocytus* is at least twelve times the internal diameter of the ovipositor through which it passes without causing noticeable expansion. The egg passes through the ovipositor like sand through an hour glass, one end emerging below before the remainder has left the body. The elastic chorion is stretched through the ovipositor by downward pointing spines on the mesal faces of the stylets. The ovipositor is capable of bending in the middle by differential stresses on the two parts of the sheath, rendered possible by a laminated bridge at the base.

The parasite feeds on host blood through a tube built from the seed coat to the host by viscous secretion from the ovipositor.

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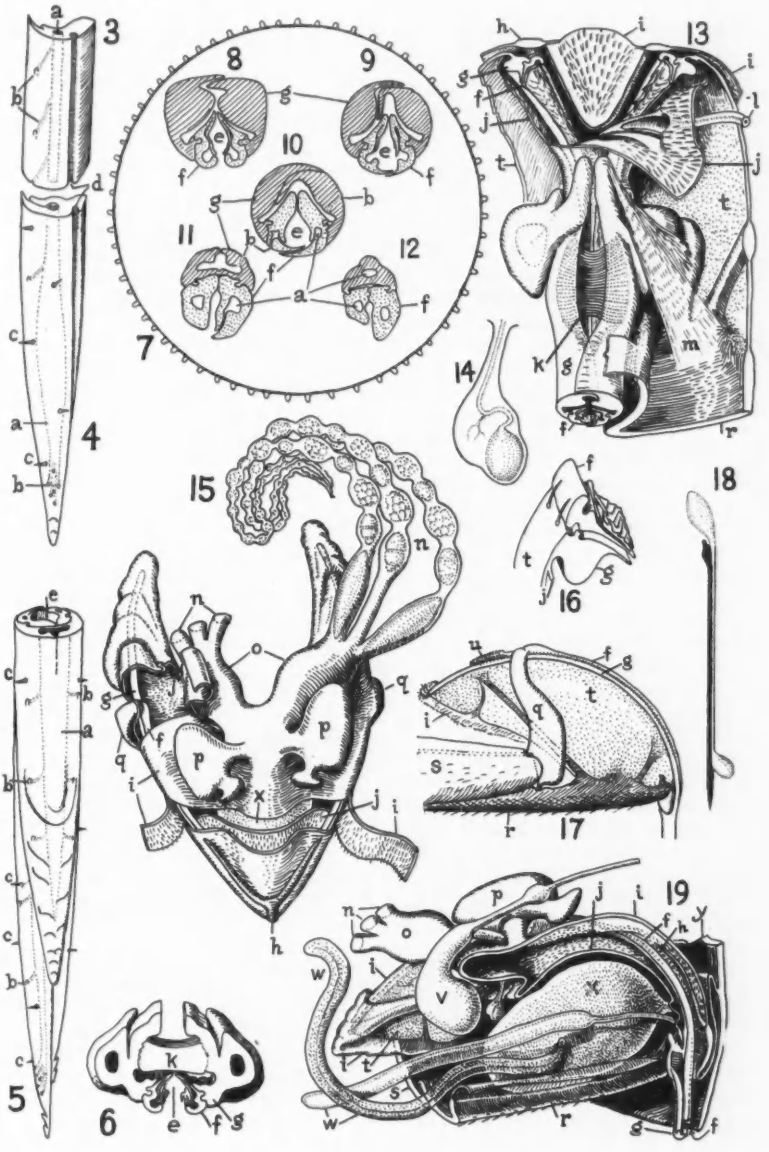
EXPLANATION OF PLATE.

Ovipositor Mechanism of *Habrocytus cerealellae*.

Fig. 3. Portion of shaft of stylet.
Fig. 4. Tip of stylet.
Fig. 5. Sheath and stylets, dorsal view.
Fig. 6. Section through base of ovipositor shaft.
Fig. 7. Section of egg chorion at greatest diameter, drawn to same scale as the enclosed cross-sections of shaft of ovipositor, (Figs. 8 to 12).
Fig. 8. Below bridge.
Fig. 9. A little below 8.
Fig. 10. Main portion of shaft.
Fig. 11. Where sheath becomes united.
Fig. 12. Tip.
Fig. 13. Dorsal or internal view of base of shaft and angle of sheath arms; ventral vaginal wall detached at left and moved to right.
Fig. 14. Spermatheca.
Fig. 15. Dorsal view of reproductive organs; ganglion and right egg tubes removed; portions of right dorsal vaginal wall laid back and middle section of dorsal and ventral walls removed exposing sac below; thickness of walls greatly exaggerated.
Fig. 16. Section of sheath arm, and stylet.
Fig. 17. Dextral view of ovipositor above shaft.
Fig. 18. Theoretical shape of egg (white) while passing through ovipositor (black).
Fig. 19. Diagrammatic mesal section of reproductive organs; egg tubes and portions of ovipositor plates removed.

SYMBOLS.

a.—canal.
b.—pores.
c.—setae.
d.—groove.
e.—egg tube.
f.—stylet.
g.—sheath (or sheath arm).
h.—Y-shaped structure
i.—dorsal vaginal wall.
j.—ventral vaginal wall.
k.—laminated bridge.
l.—gland duct.
m.—muscle.
n.—egg tubes.
o.—oviduct.
p.—bean-shaped pouches.
q.—fulcral plate.
r.—inner ovipositor plate.
s.—outer ovipositor plate.
t.—sheath arm support.
u.—spindle-shaped structure.
v.—ganglion.
w.—glands.
x.—sac.
y.—sternum.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PAUROPODA FROM NEW MEXICO.

WILLIAM A. HILTON,
Pomona College, Claremont, California.

Pauropus manus n. sp.

Length, .92 mm.; width of the head, .09 mm.; width of the body, .12 mm. The head has three rows of slightly plumed hairs on the dorsal side. There are eight in the anterior row, four in the middle row and three in the caudal row. The eye areas were not marked. The first body segment has two rows of slightly plumed hairs in two rows with two in the cephalic row and six in the caudal. To correspond to each leg pair there is a row of from six to eight hairs dorsally. All the setae are slightly plumed and short. The next to the last body division has besides the pair of long plume hairs, two rows of two each, all short and slightly plumed.

The long plume sensory hairs are of the following lengths: the first, .22 mm.; second, .28 mm.; the next, .36 mm., and the next, .33 mm.; but the last may not be complete.

Ventral hairs were not evident.

The antennae are .16 mm. long, each base is .054 mm. long, the first two basal segments are the shortest, next in length is the third basal and the fourth is the longest. The three basal segments bear short club-like hairs. The ramus bearing the globulus is a little shorter than the other. The longer flagellum of the globulus branch is nearly twice as long as the other and about as the flagellum of the other ramus. The globulus is sessile and nearly spherical.

The first two pairs of legs are the shortest. In order of shortness they are: 2–1–3–(4–8)–(5–6)–7–9. Beginning with the first they are of the following lengths: .12–.1–.13–.14–.15–.16–.14–.20 mm. There are five segments in the first two and the last pair of legs; all the others have six joints. The claws are somewhat larger than is usual. Short, slightly plumed hairs are found on the first two segments of all the legs. On the first basal segment of the last leg the hair is double. The lengths of the leg joints are as follows, beginning with the longest joint in each case: First leg: (1–2)–(3–4)–5. Second leg: (1–2–4)–(3–5). Third leg: 5–(1–2)–3–4–6. Fourth leg: 5–(1–20)–4–(3–4). Fifth leg: 5–(1–2–3–4)–6. Sixth leg: (2–4–5)–1–3–6. Ninth leg: (2–3)–(4–1)–5.

The anal plate is rather more complicated than is usual. Other distinctive features are the large number of club or slightly plumed hairs which occur in almost all parts of the body. The claws are quite marked. The second leg is peculiar in being five-jointed, a condition quite unusual in the group. This second leg is also marked by two short plume hairs on the upper side of the last joint.

This species resembles *P. indigenous* Hilton, more than any other so far described.

The above description is from a female used as the type. Several other specimens were compared. The type and several specimens were collected near Battleship Rock, New Mexico.

Pauropus nexus n. sp.

Length, .72 mm.; width of the head, .02 mm.; body at its widest, .05 mm. The head has about five rows of short knobbed hairs above. There are three on each side of the anterior margin; four in the next row; six in the next, eight in the next; and four in the most caudal on each side.

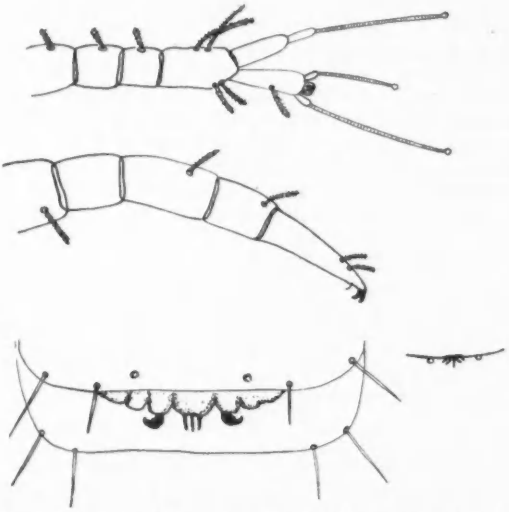

FIG. 1. At the top, antenna of *P. manus*. Next below, second leg of *P. manus*. Lowest figure, sketch of anal plate region from below, *P. manus*. Small figure at the right, anal plate region of *P. nexus* n. sp.

At the level of the first pair of legs there is a row of short clubbed hairs, four in all. At the region of the first long plume hair and at the levels of the second, third and fourth legs there are two rows of clubbed or slightly plumed hairs. The more anterior row has five, the caudal has five. Back of the fifth leg and in the region of the second plume hair there are three rows of hairs. The first of these has four hairs, the second has four and the last two. Back of the fifth leg in the second plume hair and here there are three rows of hairs. The first of these has four hairs, the second has four and the last two. Back of the third

plume hair and in the region of the seventh and eighth legs there are four rows of hairs as follows: anterior four, next two medial, next four and last two medial. Beyond the fourth plume hair at the level of the ninth legs are four hairs, two in each side. The next to the last division of the body beyond the last legs has four anterior and two posterior hairs, the last between the two last plume hairs. The very end has a long lateral hair on each side and two more caudal on each side. In the specimen it was difficult to make out the body divisions so that the above enumeration of setae has been given in relation to the position of the plume hairs and the legs. An enumeration of these in rows from back of the head and on the dorsal side is as follows: 4–4–5–5–4–4–2–4–2–4–4–2–2–4. The large plume sensory hairs were so broken that their lengths were not determined. There were no ventral hairs except at the caudal end of the animal.

The antennae were .013 mm. long. The three basal segments were of equal length and each carried a short plumed hair. The last basal joint was twice the length of any of the others. The basal segments of each of the rami were of equal length. The globulus-bearing ramus had one flagellum nearly as long as the flagellum of the other ramus, while the other flagellum of this ramus was about half this length. The globulus was small, spherical and nearly sessile.

The lengths of the legs are as follows: First, .1 mm.; second, .12 mm.; third, .12 mm.; fourth, .12 mm.; fifth, .12 mm.; sixth, .13 mm.; seventh, .14 mm.; eighth, .14 mm.; ninth, .13 mm. In order of length of joints beginning with the shortest joints, the legs are as follows: First, (2–3–4)–1–5; second, 5–(2–3)–6–4–1; third, 5–3–4–2–6–1; fourth, 5–(3–4)–1–2–6; fifth, 5–(2–3)–4–1–6; sixth, 5–(3–4)–2–(1–6); ninth, 2–3–1–4–5. The two basal segments of the first seven legs each bears a short plume hair. The eighth and ninth legs have two plume hairs on the basal segment and one on the second basal segment. These hairs are short clubs or plumes.

The anal plate has five delicate spines with two small globules near.

The type is from Battleship Rock, New Mexico.

The distinctive features are found in the anal plate, the arrangement of the hairs and their character.

Specimens were obtained in the general locality in which *P. manus* was found, which they resemble more closely than any other species. The chief differences are in the anal plate and the second leg. In the former species the second leg is five-jointed and bears two dorsal hairs on the last joint. In this species neither of these things are true.

Specimens and types of both species in the Pomona College collection, Claremont, California.

WESTERN SYMPHYLA.

WILLIAM A. HILTON,
Pomona College, Claremont, California.

Scolopendrella notella n. sp.

Length, 1 mm.

Head.—Length, .036 mm.; width, .036 mm. The head rod is forked in front and branched laterally. The head has many small hairs above, about 900 in all. There are prominent notches on the sides of the head. The ocelli are circular and .036 mm. in diameter.

Antennae.—There are 16 joints. Each has a single whorl of simple hairs. The basal segment has no long hairs, but the whorl is irregular.

Scuta.—The first scutum has about twelve short simple hairs, each on the lateral part of the scutum. There is some variation in the number of simple hairs on the free part of the scutum. There is some variation in the number of simple hairs on the free parts in various segments of the same individual as well as in other individuals. It runs from three to twelve. None of the hairs are long and there are no striations on the scutae.

Legs.—The legs are all about the same length, from .16 to .17 mm. long. All claws are well formed. There are few hairs on the legs and all are quite thick. The first leg has one or two dorsal hairs on the last joint and one on next to the last and several on the basal joints. The last leg is similar with two joints dorsally. There are not many hairs on any of the legs.

Cerci.—These are about .1 mm. long and .02 mm. wide with few hairs. They are striated near the spine at the tip. Below them on each side is a little knob with a long terminal hair and smaller ones.

Locality.—Ames, Iowa. Collected by H. B. Mills. Specimens in the collection of Pomona College.

Important Distinguishing Features.—The lateral notch in the head was found in all forms examined. The knobs with hairs between the cerci seem not so general. The mandibles have the upper tooth of the first series almost joined with the others. The antennae have simple whorls of hairs. The labrum is very hairy. The legs have no basal appendages but bear lobes on some of the basal joints. The head rod is branched behind and branched in front but not broken in the center.

Relationships.—It seems nearest to *S. vulgaris* Hansen.

Scolopendrella numexta n. sp.

Length, 1.6 mm. Body width, .13 to .18 mm.

Head.—Length and breadth, .13 mm. There are about 28 simple hairs on the dorsum of the head, counting two in the head triangle. The head rod is slender with two branches behind and with a thickened part between. It has a jointed middle portion and delicate branches near the cephalic end.

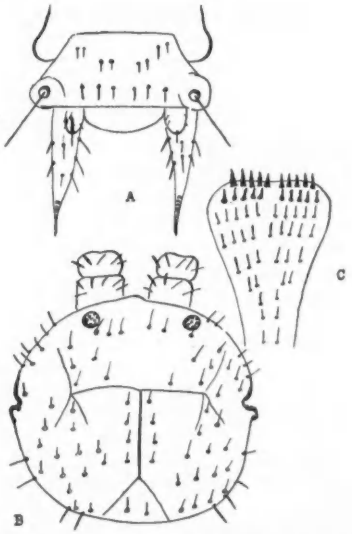

FIG 1. *Scolopendrella notella* n. sp. A, Ventral view of the caudal end. B, Head from above. C, Labium from below.

Legs.—The first leg is shorter than the others, or .14 mm.; the rest, including the last, are .2 mm. long. The claws are even and equal in size on all the legs. There were few setae on the legs, but all were covered with a fine pubescence. On the first joint this is marked with a clear smooth area of irregular size. There are about four dorsal hairs on the last joint of the last leg. This also has two ventral setae. There are several hairs on the other joints. The first leg has about four dorsal and two ventral hairs on the last joint with two or three on the next two joints.

Scutae.—These were marked, with no lateral striations and with no unusual distribution of setae.

Cirri.—These were .10 mm. by .04 mm. at the base. There were about eight cross striations near the tip. The shaft was quite hairy with more than twelve hairs on the ventral side.

Terminal Sensory Hair.—This on each side comes from a prominent lobe with two short setae near.

Locality.—Specimens were taken from the mountains of New Mexico, at about 6,000 feet altitude, not far from Battleship Rock, during the month of August, 1932.

A number of specimens were studied with the type, which is in the Pomona College collection.

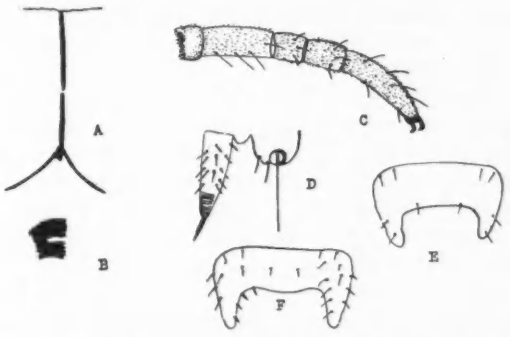

FIG. 2. *Scolopendrella numexta* n. sp. A, Head rod. B, Mandible. C, 12th leg. D, Cirrus and terminal sensory hair of one side. E, First scutellum. F, Seventh scutellum.

Important Features.—In this species there were no basal appendages on the legs. The head rod was jointed with a thickened portion behind the caudal forking. It also had delicate branches at its cephalic end. There were no lateral striations on the scutae. There were few hairs on the legs and not many on the scutae. The antennal joints had each a single ring of small setae. The mandibles had five marked teeth on each ramus.

BOOK NOTICE.

LA VIE ANIMA E, LE RAT ET SA DETRUCTION GRATUITE, by LOUIS PUEL. pp. 1–100, paper bound. Avignon, 1933.

This little volume discusses the problem of the rat from a broadly biological point of view and in the discussion draws upon our present knowledge of the spread of insect pests for some of the arguments for control of the rat. In seventeen short chapters the author reviews the problem from as many angles. It is dedicated to "Les Minestres de l'Agriculture, du Commerce, de l'Hygiène, de l'Industrie," etc., and is apparently written to engage the attention of the intelligent reader untrained in science. C. H. K.

NOTES ON ECPANTHERIA (LEPIDOPTERA, ARCTIIDAE).

WM. T. M. FORBES,
Cornell University, Ithaca, New York.

Some time ago I published a sort of revision of this genus[1] based mainly on the South American material. That revision largely passed over the North American records, because of a number of cruxes. Most of these have now been cleared up, and the present note is primarily on the Nearctic species.

I am indebted to Mr. Foster H. Benjamin, who went over the southwestern records with me, and gave me special information on the three forms limited to the Southwest; to Prof. R. O. Staig, of Glasgow, for information on the Fabrician types preserved there; and to Lord Rothschild and Dr. Karl Jordan, for opportunity to study the material at Tring, including among other things the types of *Ecpantheria oslari.*

There are four forms that reach the territory of the United States, though only one of them is really a member of the nearctic fauna.

E. scribonia Stoll. Prof. Staig's report on the Fabrician material confirms what we suspected. There is no authentic type of *deflorata*, but Mr. Staig believes that a specimen standing under a manuscript name of Fabricius is the type of that species. He sent me a figure of that specimen and it is a female of the Jamaican *E. persola*. It hardly seems possible that it is the true type, since it is hard to see how, even with an unspread specimen, the bright yellow hind wings of *persola* could be described as "posticis subtus atris, fasciis albis." It would seem that the type of *deflorata* has been lost or lost its identity, and we should probably drop the name as unrecognizable. It is certainly not our species.

On the other hand the type of *ocularia* Fabricius is reported as our species. This is entirely reasonable, and has been suspected before. There is the practical trouble that a well known South American species is now using that name; and in the interest of unambiguity I suggest that we continue to use the unquestioned name of *scribonia;* in the hope that it may be eventually made a "nomen conservandum."

[1]Annals Ent. Soc. Am. 22: 310, 1929.

E. icasia mexicana Obt. The United States specimens reported as *muzina* are of this subspecies as is to be expected. All seem to have blue bodies, none having as much orange as the form *yukatanensis*, but they vary considerably in that direction. Mr. Benjamin calls my attention to the name *sennettii* Lint., accidentally omitted from the previous paper. It is a synonym of this form.

E. confusa oslari Roth. This is undoubtedly a race of the Mexican *confusa*, distinguished by the whiter coloring with nearly wholly white hind wings, and pale filling of the eyespots on the fore wing (not dark as I had imagined). It appears to be known only from the Esperanza Ranch, below Brownsville, Texas, and is sufficiently variable there to raise a suspicion that it is hardly even a good race.

E. suffusa Schaus. *E. semiclara* Stretch appears to be a slight variant of this species and not of *oslari* or *confusa*. The species must therefore be added to the North American list.

The following notes on South American species are based mainly on material in the Zoological Museum, Tring.

E. mus pseudomus Roth. This name has priority over *castronis*. The markings are a little heavier than in typical *mus*, and the race, while slight, seems valid. It (as well as *heterogena*) is found at Rio de Janeiro. It differs by the gray-filled markings as well as basal hair, and presumably is a good species.

E. kennedyi Roth. The collar has four stripes as in *mus*, but the presence of only two pairs of stripes on the mesothorax appears distinctive.

E. persola Mosch. The male is as expected. There is no distinct alternation of size in the black spots, and there is a hair-pencil as well as the lobed anal angle, so the species is related to *orsa* and *nigriplaga*, at the foot of p. 313, Ann. Ent. Soc. Amer., Vol. XXII; the solid black spots will distinguish it from both.

E. cotyora Druce. This species shows two well marked races: (1) from Guatemala, with hind wing transparent and smoky, and the pale ground of fore wing reduced to fine lines; and (2) from Costa Rica, with hind wing much tinted with yellow, especially on inner margin, and fore wing only about two-thirds black.

Group *praeclara*. *E. laeta* Wlk. appears to be a good species

with very large anal lobe, and *praeclara* with very small lobe, but *leucarctioides* and *magdalenae* seem to intergrade. *E. m.* var. *steinbachi* may show the yellow veins but is distinguished from *praeclara* by the larger anal lobe. In *steinbachi* the blackish on the inner margin extends about half way to the base.

E. burmeisteri Roth. The type and material at Tring have the orange abdomen like a single specimen in Washington. This is evidently a normal coloring for the species. It may be differentiated from *kinkelini* by the tegulae, which have two nearly or quite separate ocelli, while in *kinkelini* they are gray with only a narrow white border.

E. melanoleuca Roth. and *obtecta* Dogn. These two forms appear to intergrade; *melanoleuca* has the brown fore tibia like *obtecta*.

E. albiscripta Dr. The female is correctly placed in the key. It has banded fore tibiae.

E. kinkelini Burm. Tring has both sexes; *aramis* is therefore at least a good form.

BOOK NOTICE.

INSECT BEHAVIOR, by EVELYN CHEESMAN. Pages 1-189; 5 text figures by the author. Published by Robert O. Ballou, New York City, 1933.

This little volume, beautifully printed and nicely bound, is a remarkable example of what can come from the pen of an author who attempts to write in a field with which she is not critically familiar. The material is divided into the following chapters: I. Antiquity of Insects; II. Simple Actions and their Origin; III. The Tyranny of Tropisms; IV. Failure of Instinct; V-VI. Instincts; Variation of Instinctive Behavior; VII. Awareness of Failure and Danger, and, X. Summary.

Chapter II opens with this, "In all animals which possess a nerve-system it is the nerves which are stimulated and through them the sense organs." Here is another, "Light for example, compels the upward growth of plants, compels certain movements and certain changes in their structure; this is known as heliotropism." After reading the passage below, the writer of this notice attempted to remember, through inheritance, some of the acts of his father when the latter was a student in college but was unable to recall anything whatever. The passage is, "A newly emerged insect is not being newly influenced by outside agents, but its behaviour is in response to the memory of what influenced its ancestors back through measureless aeons of time, etc."

Miss Cheesman has a charming style when she is describing the curious behavior of insects in the field. The present writer picked up one of her volumes on South Sea insects late one night and read it from cover to cover before going to bed. After a few pages of the present book he asked a friend to read it for him and to mark the curiosities of thought it contained. The friend was thrilled as he enjoys others mistakes.

C. H. K.

A NEW SPECIES OF PSELAPHIDAE FROM COSTA RICA.

ORLANDO PARK,
University of Illinois.

Euphalepsus liljebladi sp. nov.

(Figs. 1–11.)

One male (*holotype*).

Color.—Glistening dark reddish brown, with legs and elytra paler reddish brown. Antennae with segments 1 to 8 reddish brown with brown pubescence, segments 9 and 10 black with black pubescence, segment 11 reddish brown with golden pubescence.

Measurements.—Total length, 2.88 mm.; head, 0.6 mm. long (distal margin of labrum to posterior margin of occiput) by 0.5 mm. wide through the eyes; cervicum 0.05 mm. long; pronotum 0.55 mm. long by 0.55 mm. widest point; elytra 0.8 mm. by 1.1 mm. wide (through both elytra); abdomen 0.88 mm. long by 0.95 mm. wide; antennae total length 1.2 mm.

Head.—(Figs. 1, 4). Nearly square with well developed eyes. Front with prominent, elevated margin, ascending evenly to a median process midway between the prominent antennal tubercles. Occiput rounded anteriorly and posteriorly, sharply declivous anteriorly with a glabrous, shallow, median groove from posterior margin to a distinct angulation on anterior margin. Vertex canaliculated as follows: a deep transverse groove between antennae which communicates laterally with a pair of anterior grooves which curve between the median frontal process and the antennal tubercles, and secondly, communicates laterally with a pair of posterior grooves which curve between occiput and antennal tubercles, these posterior grooves ending behind in a pair of deep, glabrous foveae. Sides of head and posterior portion of antennal tubercles deeply and rugosely punctate; sides of occiput with small, sparsely placed, raised punctures; genae beneath eyes with four stout bristles arising from prominent, tuberculate punctures. Mandibles curved sharply at distal half, and sharply deflexed. Maxillary palpi very small; 1st segment minute, 2nd as long as 4th and slightly enlarged distally, 3rd shorter than 2nd or 4th and elongate-triangular, 4th slender, fusiform, bearing a minute bristle distally. Antennae (Figs. 2, 3) eleven-segmented, with proportions as given in Fig. 2; 1st slightly enlarged distally on internal face, 3rd shortest segment, shorter than second or fourth, 9th and 10th coarsely and confluently punctured, forming a distinct club with the 11th, the latter slightly sinuate laterally.

Pronotum.—(Figs. 1, 4). Gibbous, as wide as long, widest just behind middle, sparsely supplied with minute, raised punctures (Fig. 10).

Disk strongly, evenly elevated in center and depressed each side, then elevated to form a small, lateral swelling. A deep glabrous fovea each side, posterior to lateral swelling, these foveae connected by a shallow, glabrous groove, and the groove continuing for a short distance laterally beyond the foveae; groove without median fovea, its place occupied by a median, triangular depression. Base strongly margined, the margin with a thick median carina and a much smaller carina each side of central one.

Elytra.—(Figs. 1, 6). Nearly glabrous, with surface covered with many inconspicuous, very shallow depressions which become progressively more faint posteriorly (Fig. 11). Suture strongly beaded, the bead irregularly punctate; sutural stria entire, discal stria absent; each elytron with a bristle-bearing puncture at middle near suture, in addition to sparse, short, blunt, brownish pubescence. Base with prominent humeral swellings typical of most of the genus, these swellings continuous with wide basal margin which curves on either side of scutellum. Base of each elytron with four large foveae. Laterally, where each humeral callus joins the inflexed elytral margin there is a deep fovea. Inflexed elytral margin broad, elevated and punctured, the punctures more coarse and broken posteriorly.

Abdomen.—(Figs. 1, 5, 6, 7). Wide, arcuate, sparsely punctured and pubescent; pygidium more densely pubescent with pale hairs. Five visible tergites with proportions as in Fig. 5; 1st tergite with complete internal and external carinae, nearly parallel, with enclosed area elevated and punctured, the punctures more minute and perfect posteriorly, a distinct semicircular depression each side near basal fourth of internal carina; 2nd tergite with internal carina complete and external carina merely indicated on apical margin; 3rd tergite with one rudimentary cariniform elevation from apical margin each side; pygidium broadly emarginate apically. Six visible sternites, the first apparent only laterally and at center where it forms a prominent, table-like process between metathoracic coxae, this median process partly hidden by the rounded pad of pubescence which projects from the metasternum between coxae; 2nd sternite with median carina partly hidden beneath the median process of first segment; 3rd to 5th sternites with proportions as in Fig. 5; 6th sternite strongly emarginate apically and flattened in center.

Legs long, tarsi well-developed with a long claw and a shorter, bristle-like accessory claw as in Fig. 9. Prothoracic femora with a curved groove on the internal face (Fig. 8). Mesothoracic legs with femora bearing a strong, blunt, fasciculated spine at base, and with tibiae with a longer, fasciculated spine at posterior third (Fig. 8). Metathoracic legs normal, not spined; tibiae not at all dilated, but gradually and slightly enlarged distally, with distal, internal face strongly pubescent.

Erected upon one male (*holotype*) from Turrialba, Costa Rica; in the collection of the author. I take pleasure in

dedicating this large, distinct species to my old friend, Mr. Emil Liljeblad, of the Field Museum. The species is known only from the male, but I surmise that the probable secondary sexual characters will include the sternal pad of pubescence; the table-like process of the first sternite, which is reminiscent of males of certain North American *Rybaxis* where a similar process arises from the median posterior margin of the apparent second sternite; shape of pygidium; deeply emarginate sixth sternite; and the spined mesothoracic femora and tibiae, so typical of many species of *Euphalepsus*. With respect to such spines, the author has found the femoral spines of the male *Adranes lecontei* to serve a possible ecological function, viz., the abrasion of the host-ant brood integument (Park, 1932), and if *E. liljebladi* proves to be myrmecocolous, the male spines may be used in a similar manner. This, however, must remain unknown until the ecology of the species, or of the genus for that matter, is studied.

The genus *Euphalepsus* erected by Reitter (1883) and subsequently emended by Raffray (1890) is now restricted to batrisine species having a short, globose body, elytra with each humeral angle bearing a very prominent swelling or callosity, each elytron with three or with four foveae at base, very small maxillary palpi, and with the metasternum not carinated. Many of the species have been described from one specimen, usually the male, and in such cases the mesothoracic femora and tibiae are modified quite generally. These middle legs may have either or both the femur and tibia unarmed, or armed with large, fasciculated spines or serrations at different locations. Twenty species are listed by Raffray (1911), and since then Raffray has described two more (1917), Fletcher one (1927), and with *liljebladi*, brings the total described to twenty-four.

Euphalepsus is essentially a neotropical genus, of unknown ecology, numbering eighteen species from South America, five from Central America, and one species of very doubtful locale from Louisiana (*E. dentipes* Raffray).

The genus in the restricted sense was divided into five generic groups by Raffray (1904). This grouping needs reworking, but pending revision, the original Raffrayian arrangement is used here. This being the case, *E. liljebladi* belongs to Group II, along with four other species, and the

following group key may serve to allocate the several forms for the present:

KEY TO SPECIES OF EUPHALEPSUS IN GROUP II OF RAFFRAY.

Group Characteristics: Humeral callosity prominent, simple, not ending on elytron as a fine carina; pronotum gibbous, as wide as long approximately.

A. Vertex not strongly canaliculated by a system of grooves; third antennal segment not shorter than either second or fourth.
  B. Antennal segments nine and ten oblong or longer than wide; no small, pore-like foveae on sides of head behind eyes.
    C. Base of each elytron trifoveate.....*globipennis* Reitter
      ♂, 2 mm. Mexico.
  CC. Base of each elytron quadrifoveate.
    D. Antennal segments two to eight subquadrate to square, *humeralis* Raffray
      1 ♀, 2 mm. Colombia.
    DD. Antennal segments all oblong.....*rugipes* Raffray
      1♀? 2.25 mm. Venezuela.
  BB. Antennal segments nine and ten transverse, wider than long; each side of head behind eye, with two approximate, minute, pore-like punctures or foveae.....*panamensis* Fletcher
    1♀, 1.6 mm. Panama.
AA. Vertex strongly canaliculated (Fig. 1); third antennal segment shorter than either second or fourth.....*liljebladi* sp. nov.
    1♂, 2.88 mm. Costa Rica.

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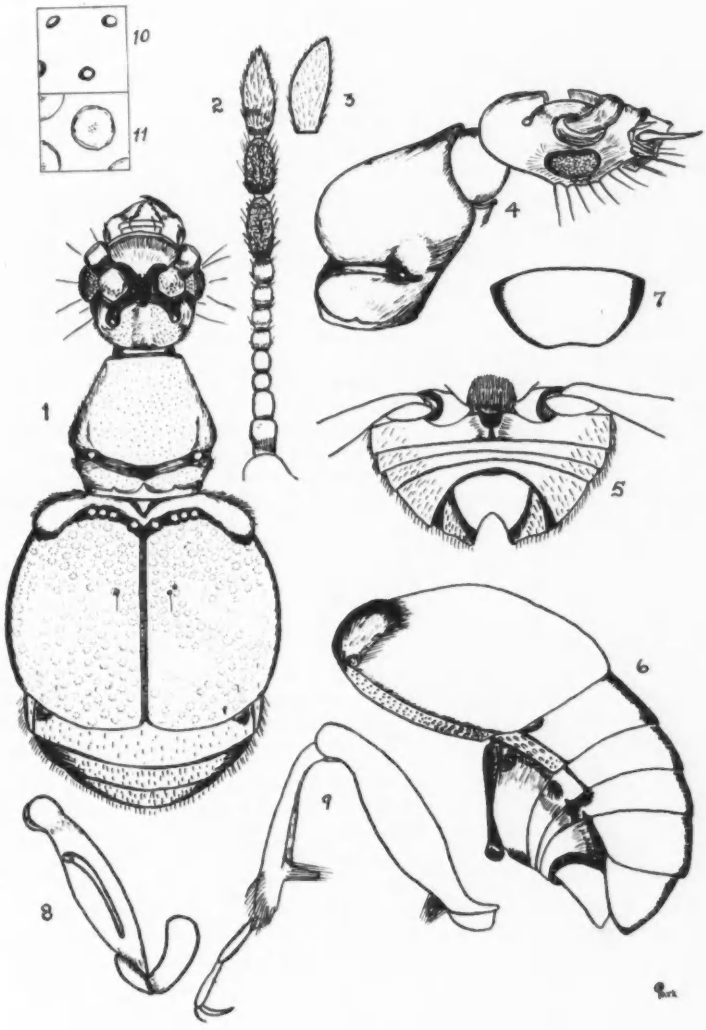
EXPLANATION OF PLATE.

Euphalepsus liljebladi sp. nov. (♂).

Fig. 1. Dorsal view of *Euphalepsus liljebladi* (× 42).
Fig. 2. Right antenna.
Fig. 3. Eleventh segment of right antenna from lateral view.
Fig. 4. Lateral view of head, cervicum and pronotum.
Fig. 5. Ventral surface of abdomen.
Fig. 6. Left lateral view of elytra and abdomen.
Fig. 7. Pygidium.
Fig. 8. Prothoracic coxa, trochanter and femur, from internal face.
Fig. 9. Mesothoracic leg.
Fig. 10. Minute, raised punctures of pronotum.
Fig. 11. Shallow depressions of elytra.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NOTES ON THE LIFE HISTORY OF THE HOUR-GLASS SPIDER.

PAUL B. LAWSON,
Lawrence, Kansas.[1]

For some years specimens of the hour-glass spider, *Latrodectus mactans* Fab. have been easy to find in the vicinity of Lawrence, particularly in the clumps of the prairie cactus, *Opuntia polyacantha*, which is quite common in some pastures. Occasional specimens are found around rocks or stumps.

WEBS.

The spiders are fairly readily located in the cactus clumps by their irregular and sparsely woven but very tough webs, which extend for about six inches to a foot in all directions in the upper part of the cactus. From the sparse upper portion of the web, a funnel-tube descends to the ground, the diameter of the tube being from one to two inches. The walls of this tube are strongly made and usually include blades of grass or portions of the cactus joints. Sometimes the tube ends at the ground surface, but usually the spider hollows out a cell in the ground, from half an inch to an inch deep, which forms the end of the tube and to which the spider retreats when disturbed.

OVIPOSITION.

Baerg[2] states that the female constructs as many as four egg cases in a season and that each contains about 300 eggs. We have never observed more than four egg cases in a single nest in the field, where they may be found in the funnel or occasionally in the upper part of the web. Several specimens kept in the laboratory, however, have constructed as many as nine egg cases as shown by the two following records:

(1) This female was found in the field July 10, 1932, with two egg cases in the funnel of her nest. In the laboratory she was kept in a pint jar and fed one large or two small grasshoppers every other day. She made egg cases on the following dates: July 17, 24; August 3, 14, 25; September 1, 10. She died on September 29.

(2) This specimen was also found in the field on July 10, but with three egg cases in her nest. In the laboratory she made egg cases on the following dates: July 27, August 3, 10, 23; September 1, 9. The last of these egg cases was very thin. On September 15 the spider seemed less active than usual, but she lived till November 14.

It seems rather clear that these two specimens were much better

[1]Contribution from Department of Entomology, University of Kansas.
[2]Scientific Monthly, xvii, p. 535, 1923.

fed in the laboratory than they would have been in the field and this undoubtedly accounts for the relatively large number of egg cases.

The number of eggs in the egg cases was found to vary greatly as would be expected by a comparison of the relative size of a number of the cases. The smallest case counted contained only 220 eggs, but no other case contained less than 400. The majority held from 500 to 700 and the largest one contained 917 eggs.

Most of the egg cases are quite regularly balloon-shaped, but with evident differences in the length of the stem. They vary from 7 to 16 mm. in length and from 6 to 13 mm. in width. Occasionally irregular cases are made, one at hand being 9 mm. wide, 20 mm. long, and is widest at the middle.

The egg cases are usually made at night during the early morning hours, though some are made during the day. Several of my students, during the summer of 1932, made observations on the construction of the egg case and all agreed that it was no use to watch the spider for this purpose during the fore part of the night, but that it was best to set the alarm clock for 12:30 A. M., since usually the females started to make their egg cases within an hour of this time. I am indebted to Mr. Walter S. Wagner for the facts in the following observation on the construction of a case.

Mr. Wagner decided to watch a female, which seemed from her swollen abdomen about ready to lay her eggs, on the night of July 17. At midnight she was quite inactive, so he set his clock for 1 A. M. At this time she was still inactive, so he set his clock for 2 A. M. However, he happened to awake at 1:32 A. M., and observed that the spider had started on her egg case. She spun quite a mass of threads across the candy jar in which she was confined, till she had a dome-like roof over her. This dome was attached to the sides of the jar and to the cheese cloth covering the jar. At 1:38 A. M. she constructed an attachment at the center of the overhanging dome of threads and by 1:47 A. M. a small, flimsy crescentic web was evident with the spider hanging below. Up to 1:55 A. M. she worked very rapidly, touching her abdomen to the edge of the crescentic web, then pulling it down, and touching the edge again further on. By this process she added rapidly to the lower edge of the original crescent and soon drew it down into a bell-shaped covering. At 1:56 A. M. she narrowed the mouth of the bell and two minutes later thrust her abdomen into the bell and laid her eggs. Oviposition took but a minute and at 2 A. M. she began closing up the bell by dabbing her abdomen across the under side. The process of covering the entire surface of the case with additional layers of silk by dabbing it all over took almost an hour and a half as compared with the half hour of work preceding egg-laying. As the case neared completion, additional stay lines were attached to it. She ceased from her labor at 3:25 A. M.

Eggs are laid in the field during the summer, the adults dying during the latter part of the summer and the early fall. One female, however, which reached maturity in the laboratory on September 1, made an egg case on October 20 and another on November 6. It is

very doubtful if this would have happened in the field in this locality. At any rate it is the general rule that individuals becoming adult one season lay their eggs during the next summer.

EMERGENCE.

Baerg states that the spiderlings leave the egg case in about ten days after the eggs are laid. We did not observe emergence before fifteen days and more frequently it was from three to four weeks before the young spiders appeared. In the latter cases, however, it was evident that the eggs had hatched quite a while before the emergence of the spiders, for the latter had begun to show the coloration characteristic of specimens in the later stages of the first instar.

Just how the spiderlings make the exit hole seems to be a question. In some cases they seem unable to make a hole and perish in the case. A number of times when the exit hole was sealed up after some of the spiderlings had emerged no new hole was made and the rest of the spiders died in the case.

IMMATURE STAGES.

While a number of specimens molted but seven times in the laboratory before becoming adults, the majority of specimens reared to adulthood molted eight times. Accordingly, this species seems to have nine instars, the adult being the ninth.

The length of the several stages depends, of course, on the food supply and other environmental factors. For example, specimens which emerged on July 12 and were not fed till July 27, did not molt into the second instar till August 14. By contrast, specimens which emerged August 3 and were fed immediately, molted into the second instar on August 8. Five days, however, was the shortest period between any two successive molts in the spider's development. Usually the period between molts for well-fed individuals was from nine to eighteen days. The total length of the cycle from emergence to adulthood may be illustrated by the following examples: (1) Emerged June 24; molted into adult September 15. (2) Emerged June 24; molted into adult September 1. (3) Emerged June 29; molted into adult October 7.

Under laboratory conditions, therefore, two to three months is required for full development. A much longer time may be taken, however, for some specimens which were taken in July, and fed normally, had not become adults by February. It seems likely, therefore, that in this latitude, some immature specimens may hibernate, though the usual practice is for the adults to live through the winter.

DISTRIBUTION OF INSTARS.

First Instar: Greatest length of fore tibia 0.4 to 0.6 mm.; usually about 0.5 mm. Cephalothorax pale watery brown except for black area on upper part of face back to ocelli. Abdomen reddish brown, marked above with broken and indistinct median white stripe, a V-shaped white stripe on base and two pairs of white oblique stripes laterally. Area

around spinnerets jet black. Legs, pedipalps and chelicerae color of cephalothorax, darkened apically.

During the life of first instar cephalothorax becomes darkened both dorsally and ventrally around margin and along dorsal median line. Abdomen becomes darker reddish brown, white stripes become more distinct, black spots or lines appear to bound mesal margins of V-shaped and lateral white stripes and dorsal white stripe becomes margined apically with dark brown or black. Legs also become darker and tibiae show signs of three dark bands. Ends of hour glass appear as white areas. Tarsi become distinctly darker. In rare cases all white stripes of abdomen become definitely bordered with black.

Second Instar: Greatest length of fore tibia 0.7 to 1 mm.; usually about 0.9 mm. Freshly emerged specimens colored about like fully matured first instars. Well colored specimens with cephalothoracic margin widely darkened, median dark line wider, white stripes of abdomen very definite and well bordered with black lines between which abdomen is brown. Hour glass white, definite in outline, central area reddish. Ends of femora and patellae dark. Ends of tibiae dark and with small dark band just beyond middle. Fore metatarsi darkened on apical half and tips of all tarsi dark. Trochanters and base of fore femora darkened and hind coxae also quite dark. All other parts of legs distinctly darker than in first instar.

Third Instar: Greatest length of fore tibia 1 to 1.4 mm.; usually about 1.3 mm. Newly emerged specimens colored about like mature second instars. In well colored specimens cephalothorax dark except for two light stripes on disc and central area light on underside. White stripes of abdomen strongly bordered with black between which are sometimes whitish mottlings. A tinge of orange usually appears on white stripe near tip of abdomen. Hour glass distinct, whitish and tinged with red. Fore legs dark except for preapical light band on femora, base of patellae, two light bands on tibiae and basal half of metatarsi. Other legs lighter, especially their femora.

Fourth Instar: Greatest length of fore tibia 1.7 to 2 mm.; usually about 1.8 mm. Well colored specimens with cephalothorax dark except for faint suggestion of light parallel lines. Abdomen jet black between white stripes, dorsal stripe distinctly though not strongly tinged with orange. Hour glass still whitish, tinged with red or orange. Epigynal area beginning to swell. Fore legs dark except for single light band on tibiae and for the basal half of metatarsi and most of tarsi. Second pair of legs dark except for light tibial band and light metatarsi and tarsi. Third pair of legs like second; fourth pair like first.

Fifth Instar: Greatest length of fore tibia 2.2 to 2.7 mm.; usually about 2.4 mm. Cephalothorax nearly entirely dark. White stripes of abdomen beginning to disappear by invasion of black spots, and usually tinged with orange, especially at base of V-shaped stripe and on entire dorsal stripe. In well marked specimens entire dorsal stripe and hour glass entirely orange. Epigynal area distinctly swollen. Legs darker than in fourth instar, sometimes entirely black except for light third femora, single bands on tibiae, and light metatarsi and tarsi. Pedipalps

dark apically. In darkening of legs first and fourth pairs color up before second and third.

Sixth Instar: Greatest length of fore tibia 3 to 3.4 mm.; usually about 3.2 mm. Cephalothorax entirely dark. Legs with but few light areas. Lateral white stripes nearly covered with black. Only base of V-shaped stripe evident.

Seventh Instar: Greatest length of fore tibia 3.6 to 3.9 mm.; usually about 3.8 mm. Cephalothorax entirely black. Lateral stripes of abdomen gone in well colored specimens. V-shaped stripe represented only by an orange spot at its base. Pedipalps entirely black for first time. Legs black except for somewhat light metatarsi and tarsi.

Eighth Instar: Greatest length of fore tibia 4.2 to 4.7 mm.; usually about 4.5 mm. Black all over except for faintly pale metatarsi and tarsi in some specimens. Orange dorsal stripe of abdomen usually very distinct and bright and consisting of two or three anterior spots and a posterior stripe.

Ninth Instar: Greatest length of fore tibia 5 to 5.9 mm. Epigynum open for first time. Red dorsal markings usually gone except for one or two red spots near apex of abdomen. Black all over except for these spots and hour glass.

The above descriptions apply only to the female. The male retains the white stripes of the abdomen throughout life and the adult also has the orange dorsal stripe conspicuously developed. The tarsi of the pedipalps of the male begin to show signs of enlargement during the third instar, increase further in size during the fourth instar, and become suddenly greatly enlarged on molting into the fifth instar. On molting into the adult they become opened and expose the copulatory organs.

The fore tibiae were used for measuring the different instars because they were found to be quite constant in length for each instar and they were easily measured. Abdominal measurements were found to be useless because of the variability caused by feeding.

METHODS OF REARING.

The egg cases of the hour-glass spider may be placed in any convenient glass container for hatching. On emergence the spiderlings will feed upon each other till in a few weeks their number will be reduced from several hundred to less than a dozen survivors, practically all of which will be females because the larger size of this sex evidently gives them an advantage in such a fierce struggle.

In order to rear specimens of both sexes, individuals may be placed in separate tubes which may be plugged with cotton. We have found celluloid tubes preferable to glass tubes because they do not break and also because the spiders are not so liable to be killed in them if exposed to the sun.

The young spiders themselves seem to be the best food in the first few days of life. However, any small insects may be used. During the summer leafhoppers are the most easily obtained of the small insects and we used them very largely, although small Diptera and

Hemiptera were often used. In the winter fruit flies are most easily kept on hand. During the summer season the specimens were fed about every other day.

As the spiders grow, larger and larger insects may be fed them so that during the last three or four instars they may be fed such insects as grasshoppers and other Orthoptera.

For rearing fruit flies the writer has found a high bell glass with an open mouth about an inch in diameter very convenient for rearing and handling fruit flies. This jar is used lying on its side so sufficient air can enter through the cheese cloth fastened over the wide open end. If stood on end too much moisture gathers in the jar and the development of the flies is hindered. A short glass tube, about half an inch in diameter, is inserted in the narrow mouth of the jar by means of a perforated cork, and a short piece of rubber tubing is attached to the tube and closed with a clamp. Since the fruit flies are positively phototropic they rush into the glass tube if it is directed towards the light while the jar is covered with a black cloth. Feeding then simply consists of holding the spider's tube over the end of the rubber tube, opening the clamp, and letting the desired number of fruit flies enter the spider's tube.

During the summer it did not seem necessary to give the spiders any water, at any rate they got along without any. In the winter, however, water seems to be needed and was introduced into the tube by a pipette about every two weeks.

A large series of specimens which were growing and molting regularly in Kansas, were taken along by the writer on a trip to Colorado for about ten days. In the mountains they practically quit molting, but resumed it again on their return to the plains. Whether this was due to the disturbance of travel or to the change in altitude from less than 1,000 feet to one of over 9,000 feet, is not clear.

The writer has never tried to rear any Arthropods, which are more easily handled and which are easier to rear because of their hardiness. With very ordinary care, few specimens die, so that one may expect to raise to maturity the large majority of any series of newly-hatched specimens. When the tubes are opened for the insertion of food and water, the spiders very rarely attempt to escape, never attempt to attack one, but run instead to the closed end of their tubes.

DISTRIBUTION.

Latrodectus mactans has previously been reported from the West Indies and South and Central America. In the United States it has been reported from the southern states and from the northern states of Indiana, Ohio, Pennsylvania and New Hampshire. Professor Eugene F. Powell, of the University of Nebraska, writes that it has been reported from Nebraska and that he has seen specimens in Colorado. Dr. George List and other Colorado entomologists have frequently seen it in Colorado. Dr. Vasco Tanner says he has seen it in Utah. Mr. Cornelius Philip writes that there is a considerable infestation of the species around Hamilton, Mont. Mr. E. J. Newcomer writes that it

is quite common in central Washington. Dr. H. B. Hungerford took a specimen in the summer of 1932 at Cheboygan, Mich.

Since this spider has been taken in such northern states as Washington, Montana, Utah, Michigan and New Hampshire, it is evident that it must occur throughout the United States and up into Canada. The writer would like to suggest, however, that in the north we may be dealing with a different species or variety of the spider, for spiderlings hatched from the eggs of the specimen taken in Michigan by Dr. Hungerford were quite differently marked from the spiderlings of the Kansas form. Unfortunately these Michigan spiderlings were so nearly dead when given to the writer that he was not able to revive and rear them.

BOOK NOTICE

BIBLIOGRAPHIA ODONATOLOGICA, by Dr. Erich Schmidt. Liferung I (Seite 1–116 und Tafel I), 1933. Published by Fritz Wagner, Vienna, Austria, R M. 10.65.

This is the first part of a very elaborate bibliography of the literature on Odonata by Dr. Erich Schmidt, of Berlin. For twenty years Doctor Schmidt has been a recognized specialist on this order of insects. This work brings together in a detailed list the titles of all scientific writings that discuss or mention dragonflies. The bibliography includes the usual titles of scientific publications but also titles of works of travel that mention or give random notes. The compilation has been done with great thoroughness, as Doctor Schmidt has called on students the world over for rare items which have been shipped to him in Germany to be checked and returned. The writer can attest the courteous promptness with which these items have been consulted and returned by registered post.

This is the most comprehensive work in the field of Odonatology since the publication of "A Synonymic Catalogue of Neuroptera Odonata," by Kirby in 1890. It has been very much needed. The writer wonders, if it might not have been more helpful, if it were a little less complete. However, the material is so well organized that little difficulty will be found in using the work.

The first "Liferung" covers names from Aaron to Dobson and in the 116 pages it is estimated there are 1500 titles. This shows the extent of the work as the writer in discussions with E. B. Williamson in 1920 estimated that a library of about 2500 titles was needed in doing thorough systematic work on dragonflies.

At about this same date work had been started on such a bibliography by B. Elwood Montgomery of Purdue University, who used the material in the library of E. B. Williamson. On hearing of Doctor Schmidt's work along the same lines Mr. Montgomery turned his manuscript and notes over to the latter.

This problem of literature is becoming a very serious one as systematic work develops. It takes years and a continuous investment to acquire and keep up to date a library on as small a group as the Odonata. In the future, work will have to center more and more around Museums and Universities.

The largest private library on the subject of dragonflies is that of the late Mr. E. B. Williamson which is now in the Zoological Museum of the University of Michigan. This with his very large collection, particularly of North and South American species, will make Ann Arbor a place visited by specialists on this group for years to come. The next library in importance is that of Professor P. P. Calvert at the University of Pennsylvania, in Philadelphia, and in the same city is Doctor Calvert's collection, second in importance in this country, which is now deposited in the Philadelphia Academy of Natural Sciences. The writer's library is probably third in size but is not associated with an extensive collection. The other centers where libraries are associated with collections are Toronto University (Prof. E. M. Walker), the U. S. National Museum, the Museum of Comparative Zoology (the Hagen collection), the American Museum of Natural History, and Cornell University (Prof. James G. Needham). C. H. K.

CONTRIBUTIONS TO THE MORPHOLOGY OF THE NERVOUS SYSTEM OF THE MATURE LARVA OF PAPILIO POLYXENES FAB.

(Lepidoptera, Papilionidae.)

HOWARD H. HILLEMANN,
Milwaukee, Wisconsin.

Investigations on the nervous system of *Papilio polyxenes*, the dill-worm, were begun in the summer of 1932. Since our present knowledge of the morphology of the family *Papilionidae* is somewhat scant, the objective held in view was to contribute a description of the nervous system of a member of this family. The study here presented is confined to the central and sympathetic systems.

In reviewing the literature dealing with the morphology of the nervous system in insects, a number of pertinent papers were found, and are listed in the bibliography. Among these is the paper on the anatomy of the tomato-worm larva, *Protoparce carolina* (*Sphingidae*), by Alvah Peterson, which appeared in 1912. This paper includes a description of the nervous system in *Protoparce*, and served as a model in working out the nervous system in the dill-worm.

The nervous system of *Papilio polyxenes* as in all insects, is ventral in position and lies for the most part along the meson. It consists morphologically of ganglia, nerves, commissures and plexi. There are thirteen mesally located ganglia: two in the head, one in each of the three thoracic segments, one in each of the first six abdominal segments, a double ganglion in the seventh, and none in the eighth. Nerves extend outward from these ganglia, while commissural cords unite the ganglia so that the system appears as a slender cord with definite enlargements at segmental intervals. In some places nerves extend from the commissures also. In addition to those mentioned, there are six smaller ganglia with their nerves, which constitute the two sympathetic systems of the head. Sympathetic nerves and plexi are associated also with most of the other ganglia. The lower or ventral sympathetic system will not be discussed under a separate heading, for it is more convenient to speak of the sympathetic plexi, median and transverse nerves which

make up this system when describing those ganglia with which they occur.

HEAD GANGLIA.

Supraoesophageal ganglion—(Fig. 1, H. lg.). The supraoesophageal ganglion is the largest ganglion of the entire nervous system, with a transverse diameter which is twice that of its dorsoventral diameter. Its diameter dorsoventrally and cephalocaudally is approximately the same. It consists of two distinct lobes which unite along the meson. The ganglion lies in the front of the head capsule and on the cephalic surface of the dorsoventrally directed pharynx. Several nerves arise from this ganglion. They are:

Crura cerebri (cc.). Two large cords, the crura cerebri, comparable with the commissures uniting the other ganglia, arise from the middle of the laterocaudal margins of the supraoesophageal ganglion and proceed dorsocaudally around either side of the pharynx to form the circumoesophageal ring by their union with the suboesophageal ganglion.

Optic nerves (on). The optic nerves arise from the anterolateral angles of the ganglion, and proceed in a cephalolateral direction for almost one-half their length; they then curve and proceed directly laterad to the ocelli, where a small branch is sent to each ocellus. The bend in the nerves is due to the presence of large muscle bands which obstruct a straight path.

Antennal nerves (atn). Arising from the ganglion ventromesal to the origin of the optic nerves and just cephalad and adjacent to the origin of the clypeolabral nerves, are the antennal nerves. They extend cephaloventrad and laterad and innervate the antennae.

Clypeolabral nerves (cllrn). This pair of nerves arises most caudally of all nerves of this region of the ganglion. These nerves give off two mesally directed branches. The first arises from a point ventral to and opposite the frontal ganglion, and proceeds caudomesally and ventrally. The second branch arises at a point twice the distance of the first, from the base of the clypeolabral nerve, and proceeds caudomesally and somewhat dorsally, to join the ventrolateral branch of the frontal nerve of the vagus system. The clypeolabral nerves also give off three caudolaterally directed branches: the first arises directly opposite the first mesal branch; the second arises farther ventrad and terminates in a ganglion-like structure on the lateral side of the pharynx opposite the labrum, giving off in turn four small nerves; the third branch arises slightly farther ventrad, and from a point just dorsal to the second mesal branch, being directed cephalolaterally and ventrally.

Tracheation. A tracheal branch (tb) enters the supraoesophageal ganglion just dorsal to the insertion of the crura cerebri. This trachea arises from a branch coming from the spiracle in the first thoracic segment.

Suboesophageal ganglion—(Fig. 2, H. 2g.). The suboesophageal ganglion is considerably smaller than the preceding one. It lies on the caudal side of the pharynx in a plane ventral to that of the supraoesophageal ganglion. The following nerves are found to arise from it:

Crura cerebri (cc). Since the crura cerebri were already described under the supraoesophageal ganglion, it is only necessary to add that they unite with the suboesophageal ganglion in the cephaloventral portion of its lateral margins.

Suboesophageal commissure (sc). Arising from the inner surface of the middle of the crura cerebri, and proceeding caudoventrally and mesally to form a ring about the pharynx, are the cords of the suboesophageal commissure. As suggested by Peterson (17), these cords may be homologous to the connective nerves of the thoracic commissures which have here come together to form a ring about the pharynx, instead of proceeding independently in opposite directions.

Pharyngeal nerves (pn). Two very small pharyngeal nerves arise in close proximity to each other from the suboesophageal commissure on either side of the meson. They were found to innervate the pharyngeal muscles.

Mandibular labial nerves (mdlbn). Arising adjacent to the crura cerebri, slightly caudal and mesal to them, and extending ventrad are the mandibular labial nerves. They shortly divide into two branches, the mandibular nerve and the labial branch nerve. The mandibular nerve (mdn) is a large thick branch extending cephaloventrad, innervating the muscles of the mandible. The labial branch nerve (lbb) is a fine branch coming off the mesal side of the mandibular labial nerve and extending caudoventrad, innervating the muscles of the labium.

Maxillary nerves (mxn). These arise near the mandibular labial nerves, ventrolateral and caudal to the latter. They extend ventrolaterad, and give rise to several branches which innervate the maxillae.

Labial nerves (lbn). A pair of nerves arise from the middle of the caudoventral surface of the ganglion between the meson and the lateral margin, and extend ventrocaudad. Peterson, in his paper already referred to, calls this pair of nerves the "unidentified nerves," since, as he states, he was unable to determine what they innervate. But in this study, these nerves were definitely traced to the muscles of the labium, and are therefore a second set of labial nerves. Hence, what are called labial nerves in this paper, correspond to the "unidentified nerves" of Peterson, and what are called labial branch nerves here, correspond to those nerves which Peterson calls "labial nerves."

Ventral nerves (v). The ventral nerves arise from the middle of the lateral margin of the suboesophageal ganglion. They extend in a dorsocaudal direction, and innervate the caudal part of the head.

Tracheation. At a point just ventrocephalic and adjacent to the origin of the ventral nerves, the tracheal branches (tb) enter the ganglion, arising from a transverse trachea (tt) which runs directly across the middle of the dorsocephalic surface of the ganglion.

SYMPATHETIC SYSTEMS OF THE HEAD.

Vagus or unpaired sympathetic system—(Fig. 1). This sympathetic system consists of only two small ganglia and four nerves. They are as follows:

Arched nerves (ar). Arising from the ventrolateral area of the supraoesophageal ganglion, and proceeding cephaloventrad and mesad to form an arch, are the arched nerves. These nerves meet on the meson to form a small ganglion.

Frontal ganglion (fg). The frontal ganglion consists of the fused mesal part of the arched nerves. Two nerves arise from it.

Frontal nerve (fn). After arising from the middle of the ventral margin of the frontal ganglion, the frontal nerve extends for a short distance ventrad, and soon forks into two branches which proceed ventrolaterad.

Recurrent nerve (rn). On the opposite side of the frontal ganglion, on the meson, is found the recurrent nerve. It extends from the ganglion dorsad, beneath the aorta, and lies on the dorsal surface of the pharynx and oesophagus. The nerve follows caudally along the pharyngeal flexure. Continuing, the nerve gives off lateral branches which are usually paired, and which innervate the muscles of the Pharynx and oesophagus. These branches also supply the aorta. In Figure 1, the recurrent nerve is drawn thicker in proportion to the other structures than it actually is.

Vagus ganglion (vg). Near the posterior end of the oesophagus, the recurrent nerve forks and gives rise to two branches. At this point of bifurcation the recurrent nerve is somewhat thickened, thus forming the minute vagus ganglion.

Stomogastric nerves (st). The two branches proceeding from the vagus ganglion in a caudolateral and ventral direction around the outer surface of the oesophagus, are the stomogastric nerves. These nerves innervate the caudal portion of the oesophagus, and give rise to smaller lateral branches as they proceed.

Paired sympathetic system—(Fig. 1). In this system are found two pairs of ganglia and four pairs of nerves, as follows:

Lateral nerves (l). Lying ventromesal to the entrance of the tracheae, a pair of small lateral nerves enter the supraoesophageal ganglion. They proceed dorsally and caudally, each terminating in a sympathetic ganglion at the side of the pharynx.

Anterior lateral ganglia (alg). This pair of sympathetic ganglia, the anterior lateral ganglia, are irregularly ovoid in shape, and are tilted with their ends pointing anterodorsally and posteroventrally. In addition to the lateral nerves which enter in at their anterodorsal tips, the ganglia give rise to a pair of frontolateral nerves, and to a pair of nerves extending caudally from their posteroventral ends. These same ganglia also give rise to another pair of nerves which may be designated as postlateral nerves.

Postlateral nerves (pln). At the point where the lateral nerves enter the anterior lateral ganglia, the postlateral nerves also enter, arising from the recurrent nerve of the vagus system.

Frontolateral nerves (fl). The frontolateral nerves arise from the middle of the ventral margins of the anterior lateral ganglia. They continue cephaloventrad to the caudolateral aspects of the supra-oesophageal ganglion, each sending into the ganglion a short stub, and

proceeding farther ventrad and laterad into the head, each giving rise to several distal branches.

Lateral commissures (lc). A pair of short nerves, the lateral commissures, arise from the ventral margins of the anterior lateral ganglia near their posteroventral ends, and connect them with a second pair of smaller sympathetic ganglia, lying just caudal to the first.

Posterior lateral ganglia (plg). This second pair of sympathetic ganglia are the posterior lateral ganglia, and are also irregularly ovoid in shape, but only half the size of the anterior lateral ganglia. A few small nerves were found to arise from the margins of each.

THORACIC GANGLIA.

Prothoracic ganglion—(Fig. 3, T. lg.). The prothoracic ganglion is the simplest of all the ganglia of the central nervous system, inasmuch as only three pairs of nerves emanate from it. The total absence of lateral nerves in all the thoracic ganglia is worthy of special mention. This finding is quite the opposite of that of Peterson for *Protoparce carolina*. There are no median or transverse nerves in this ganglion, as there are in the other two thoracic ganglia as well as in the abdominal ganglia. Also, there is no subconnective nerve present in this or in the other two thoracic ganglia. The following nerves however, are present:

Ventral nerves (v). The ventral nerves arise from the middle of the lateral margins of the ganglion, proceed directly laterad for a very short distance, and then divide into three finer branches: one branch running directly laterad, a second directly laterocaudad, and a third ventrad and slightly caudad. The lateral branch is thicker than either of the other two.

Connective nerves (cn). These arise from the commissural cords between the suboesophageal and prothoracic ganglia, midway between the ganglia, proceed directly laterad a distance about equal to the length of the commissure, and then give rise to several smaller branches.

Tracheation. A fine tracheal branch (tb) enters the ganglion just anterior to the trunk of the ventral nerve, arising from a transverse trachea (tt) located slightly anterior and ventral to the ganglion.

Meso- and Metathoracic ganglia—(Fig. 4). These ganglia are slightly larger and closer together than the abdominal ganglia. The cords of the commissures between the prothoracic and mesothoracic ganglia, and between the mesothoracic and metathoracic ganglia are separate throughout, lying farthest apart in the anterior two-thirds of their length, thus enclosing a diamond-shaped area. The commissure between the metathoracic ganglion (T. 3g.) and the first abdominal ganglion is considerably shorter than any other thus far considered, and its cords are separate in the posterior two-thirds of its length. The median and transverse nerves in these two ganglia form the ventral sympathetic system in this region. In the abdominal ganglia, there is an additional sympathetic web-like plexus which is not present here.

Connective nerves (cn). These nerves arise from the lateral angles of the diamond formed by the cords of the commissure, and extend laterad and slightly cephalad. The lateral angles of the diamond-shaped areas are in some cases, not directly opposite, so that the right connective nerve may arise slightly anterior to the left connective nerve, or vice versa.

Ventral nerves (v). The ventral nerves arise from the middle of the lateral margin of the ganglion and proceed laterocaudad and ventrad from the metathoracic ganglion and laterocephalad and ventrad from the mesothoracic ganglion. They innervate the ventral portions of the thorax.

Median nerve (m). An unpaired median nerve arises from the middle of the posterior margin of the ganglion and proceeds directly caudad between the two cords of the commissure, about one-third the length of the cord, then bifurcating into two transverse nerves.

Transverse nerves (tn). From the bifurcation of the median nerve, the transverse nerves proceed posterolaterad until they meet a branch given off anteriorly by the connective nerves; they then proceed directly laterad and receive another branch from the connective nerve.

Postmedian nerve (pm). A very fine nerve, which may be designated as the postmedian nerve, arises from the transverse nerve very near the point of bifurcation of the median nerve. It proceeds directly caudad and enters the middle of the anterodorsal surface of the ganglion.

Anterior nerve (an). Another small nerve, which may be called the anterior nerve, arises from the postmedian nerve at a point about one-fifth its length from the junction of the postmedian nerve with the transverse nerve. It proceeds directly cephaloventrad and soon breaks up into two fine branches which continue ventrad and slightly cephalad.

Tracheation. A little anterior to the origin of the ventral nerves, tracheal branches (tb) enter the ganglion. These branches arise from a transverse trachea (tt) located a distance the length of the ganglion, anterior and ventral to it.

ABDOMINAL GANGLIA.

First abdominal ganglion—(Fig. 5, A. lg.). Since there is no essential difference between the first six abdominal ganglia, a description of the first abdominal ganglion will suffice for all six. The abdominal ganglia are among the smallest ganglia in the central nervous system. Sympathetic plexi are found closely associated with all the abdominal ganglia except the eighth, and lateral nerves are present in all eight.

Commissure (c). The commissure consists of two cords throughout, showing a median furrow or being separated in places but not far apart. The commissure leaves the posterior end of the ganglion and proceeds posteriorly to unite with the second abdominal ganglion at its cephalic end.

Lateral nerves (l). Lateral nerves arise from the cephalic part of the lateral margins of the ganglion, and proceed at first cephalolaterad for a short distance and then curve back to run directly laterad. They were traced to the laterodorsal region of the body wall.

Ventral nerves (v). The ventral nerves arise caudal to the lateral nerves at the lateral margin of the ganglion, slightly posterior to the middle of the ganglion, and somewhat ventral to the origin of the lateral nerves. They extend laterocaudad and innervate the ventral parts of the body segment.

Median nerve (m). The median nerve appears to arise at the point of bifurcation of the commissure, but upon careful separation of the cords of the commissure anterior to their point of bifurcation, it was found actually to arise directly from the ganglion as a separate nerve between the two commissural cords. The median nerve proceeds caudad, dorsal to and between the two commissural cords. Anterior to the ganglion about one-third the length of the ganglion, the median nerve divides, thus giving rise to the two transverse nerves.

Transverse nerves (tn). Leaving the median nerve, the transverse nerves proceed caudolaterad a distance equivalent to about one-third the length of the ganglion; they then bend abruptly and proceed anterolaterad and ventrad. Immediately beyond this bend, they give rise to a branch which proceeds laterocephalad and ventrad.

Postmedian nerve (pm). From the point of bifurcation of the median nerve, a very fine postmedian nerve also arises and proceeds directly caudad to enter the anterodorsal surface of the ganglion.

Sympathetic plexus (px). Between the ganglion, the lateral nerve, and the transverse nerve, the web-like sympathetic plexus is found, extending outward a distance equivalent to about the length of the ganglion. From the end of the plexus, the transverse nerve proceeds anterolaterally.

Tracheation. A tracheal branch (tb) enters the ganglion from a ventrolateral and slightly caudal direction, and is inserted into the side of the ganglion immediately anterior to the origin of the ventral nerves. Proximally, the tracheal branches arise from a transverse trachea (tt) located a short distance posterior and ventral to the ganglion.

Seventh and eighth abdominal ganglia—(Fig. 6, A.; 7, 8, g.). This fused ganglion is the most posteriorly located ganglion of all, and is found in the seventh abdominal segment. The ganglion is double, resulting from the fusion of the ganglia of the seventh and eighth abdominal segments and showing no visible commissure. The anterior half of this fused ganglion, which represents the seventh abdominal ganglion, is quite similar to the first six abdominal ganglia and therefore needs no further comment. In the posterior half of this elongated ganglion, however, which half represents the eighth abdominal ganglion, the nerves are lengthened and their directions changed. The points of origin of these nerves are also somewhat different. There is no sympathetic plexus or postmedian nerve for this ganglion.

Lateral nerves (l). The lateral nerves leave the side of the ganglion just anterior to the posterolateral angles and proceed posteriorly and somewhat laterad, soon dividing into two branches which proceed farther caudad and ventrad to innervate the lateral and ventral portions of the eighth abdominal segment.

Ventral nerves (v). These leave the ganglion at the posterocaudal angles and proceed posteriorly and somewhat laterally, almost parallel with the trunk of the lateral nerves, far into the caudal portion of the eighth abdominal segment, where they send off branches innervating the caudoventral portions of the segment.

Median nerve (m). The median nerve arises from the center of the dorsal surface of the double ganglion, proceeds a short distance directly caudad, and then bifurcates at a point slightly beyond the posterior end of the double ganglion.

Transverse nerves (tn). From the point of bifurcation of the median nerve, the transverse nerves proceed directly caudolaterad for a considerable distance before giving rise to branches which innervate the ventrolateral region of the eighth abdominal segment.

Tracheation. Two fine tracheal branches (tb) follow along the main trunks of the lateral nerves and enter tha ganglion apparently with the lateral nerves but ventral to them. These fine branches have their origin in a transverse trachea (tt) located far posterior to the ganglion, thus giving great length to the tracheal branches.

SUMMARY.

1. The "unidentified nerve" of Peterson was found to innervate the labium, and was accordingly called the labial nerve; Peterson's "labial nerve" (branch of the mandibular labial nerve) was designated as the labial branch nerve.

2. Lateral nerves were found to be entirely wanting in the thoracic ganglia.

3. The prothoracic ganglion lacks the subconnective nerve described in *Protoparce* besides other nerves present in the other two thoracic ganglia.

4. Several fine nerves, previously unnamed, were designated as the anterior nerve, postmedian nerve, and postlateral nerve.

5. Sympathetic plexi were found only in the first seven abdominal ganglia.

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EXPLANATION OF PLATE.

Fig. 1. Dorsal aspect of supraoesophageal ganglion, showing nerves, tracheation, the paired sympathetic system and the vagus (unpaired sympathetic) system.

Fig. 2. Dorsal aspect of suboesophageal ganglion, showing nerves and tracheation.

Fig. 3. Dorsal aspect of first thoracic ganglion, showing nerves and tracheation.

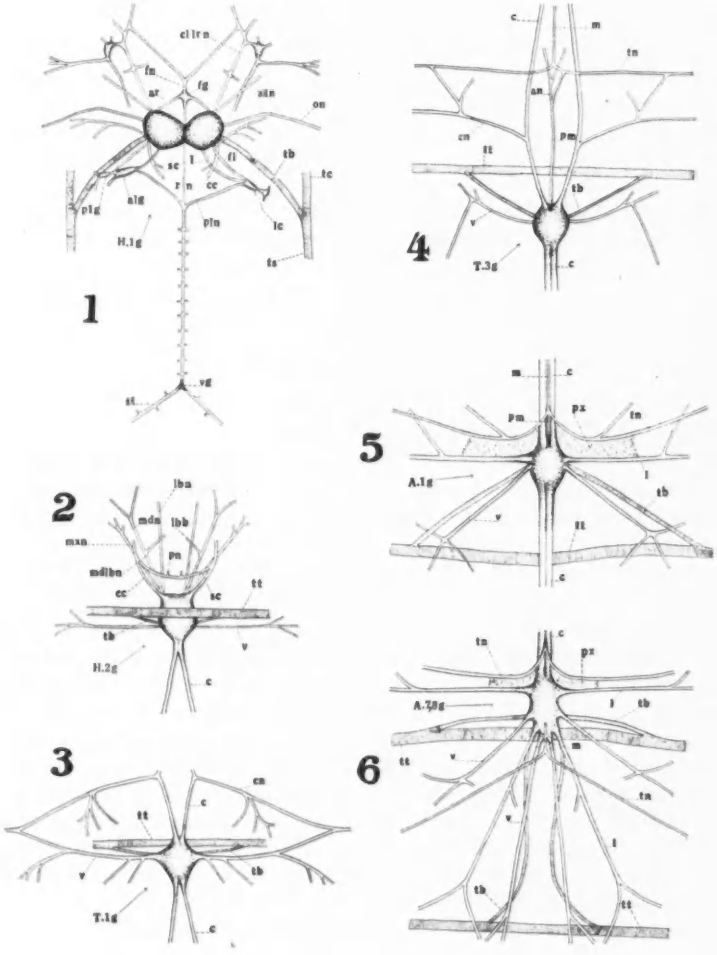
Fig. 4. Dorsal aspect of third thoracic ganglion, showing nerves and tracheation.

Fig. 5. Dorsal aspect of first abdominal ganglion, showing nerves, plexi and tracheation.

Fig. 6. Dorsal aspect of the fused seventh and eighth ganglion, showing nerves, plexi and tracheation.

LIST OF ABBREVIATIONS.

alg.....anterior lateral ganglion.
an.....anterior nerve.
ar.....arched nerve.
atn.....antennal nerve.
A. 1g.....abdomen, first ganglion.
A. 7, 8g....abdomen, seventh and eighth ganglia.
c.....commissural cord.
cc.....crura cerebri.
cllrn.....clypeolabral nerve.
cn.....connective nerve.
fg.....frontal ganglion.
fl.....frontolateral nerve.
fn.....frontal nerve.
H. 1g.....head, first ganglion.
H. 2g.....head, second ganglion.
l.....lateral nerve.
lbb.....labial branch nerve.
lbn.....labial nerve.
lc.....lateral commissure.
m.....median nerve.
mdlbn.....mandibular labial nerve.
mdn.....mandibular nerve.
mxn.....maxillary nerve.
on.....optic nerve.
plg.....posterior lateral ganglion.
pln.....postlateral nerve.
pm.....postmedian nerve.
pn.....pharyngeal nerve.
px.....plexus.
rn.....recurrent nerve.
sc.....suboesophageal commissure.
st.....stomogastric nerve.
tb.....tracheal branch suppling ganglion.
tc.....tracheal branch to front of head.
tn.....transverse nerve.
ts.....tracheal branch from spiracle in first thoracic segment.
tt.....transverse trachea.
T. 1g.....thorax, first ganglion.
T. 3g.....thorax, third ganglion.
v.....ventral nerve.
vg.....vagus ganglion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SOME RELATIONS BETWEEN ALTITUDE AND THE DISTRIBUTION OF ACRIDIDAE IN COLORADO.

GORDON ALEXANDER,
University of Colorado.

It is a well known fact that mountain ranges extending in a north-south direction carry boreal species far south of their latitudinal range at sea-level. That altitude is of pronounced effect in a very limited area is not so widely known. Such an effect was, however, clearly demonstrated in a series of studies undertaken during the summer of 1932 by a graduate class in Animal Ecology at this institution. The results here reported are due to the cooperation of Mr. Lawrence Jones, my assistant, and Miss Nora O'Mara and Messrs. H. B. Perrin, A. L. Oder, R. E. Rodock, and D. F. Crain, members of the class, all of whom assisted in the collection of data.

Two areas, a little less than a mile apart, were contrasted. The difference in altitude was almost exactly 900 feet. At each station two plant communities were selected as places for collections, these occurring at both elevations. These were (a) short grass, and (b) open forest (*Pinus scopulorum*). The seven collectors were divided into two groups, one of three, the other of four, the two groups collecting simultaneously at the two different altitudes. Three collecting trips were made; on the first and last trips the group of three worked at the lower area, on the second trip the larger group collected there. Each group collected for about one hour in grassland, and one hour in open forest, on each date. In other words, there were twelve, one-hour, group collections. Individual differences in collecting ability are partly eliminated by adding together the collections of all individuals in one group. Consistent total differences, especially of some magnitude, may, of course, be considered of real significance.

The results have been summarized in the accompanying three tables. Table I contrasts the Acrididae taken in short grass at the two elevations, and Table II contrasts those of the open forest. In both these tables the collections are separated according to dates. The collections made on different dates

are all combined in Table III, which summarizes all observations. For simplicity, the authorities for specific names are given only in Table III.

It is, of course, important to remember that the dates of such collections are quite significant; although workers have not always kept this fact in mind. On a given date the contrast may be very striking, but two weeks or a month later it may have disappeared. Less thorough collecting, both earlier and later

TABLE I.

ACRIDIDAE COLLECTED IN SHORT GRASS, AT TWO DIFFERENT ELEVATIONS.

SPECIES	NUMBER OF SPECIMENS[1] COLLECTED					
	June 23		June 28		July 14	
	5800′	6700′	5800′	6700′	5800′	6700′
Amphitornus coloradus	.....	.....	.....	.....	14	1
Eritettix tricarinatus	5	.....	1	1	2	.....
Chrysochraon abdominalis	.....	.....	.....	.....	2	.....
Gomphocerus clavatus	26	2	8	4	27	2
Ageneotettix deorum	.....	.....	.....	.....	9	10
Arphia conspersa	1	.....	.....	8	.....	.....
Camnula pellucida	.....	.....	1	9	1	37
Xanthippus corallipes leprosus	.....	2	.....	.....	.....	.....
Spharagemon collare	.....	.....	.....	.....	2	.....
Trachyrhachis kiowa kiowa	.....	.....	.....	4	17	3
Circotettix rabula rabula	.....	.....	.....	.....	.....	5
Hesperotettix viridis viridis	.....	.....	.....	.....	53	.....
Melanoplus dodgei incultus	.....	.....	1	1	1	.....
M. bivittatus	.....	.....	1	1	4	1
M. dawsoni	.....	.....	.....	.....	1	.....
M. confusus	.....	.....	.....	.....	3	.....
M. infantilis	.....	.....	.....	3	.....	24
M. occidentalis occidentalis	1	.....	2	1	.....	.....
M. mexicanus mexicanus	.....	.....	14	10	60	2

than that reported, emphasized the importance of this statement; for example, something over a month after the last collection here mentioned, *Hesperotettix viridis* was found not only at the 6,700-feet elevation, but also a thousand feet higher than that.

Two of the species collected, *Arphia conspersa* and *Gomphocerus clavatus*, are distributed from the base of the foothills to

[1]The numbers in the tables include adult specimens only.

elevations above timber-line, appearing later in the season at higher elevations. The dates of collections are extremely important in a consideration of the distribution of these forms. Within the limited area studied, dates are important for the same reason for *Hesperotettix viridis*, *Melanoplus bivittatus*, *M. dawsoni*,[2] and *M. mexicanus*, although none of these occurs at extremely high altitudes.

TABLE II.

ACRIDIDAE COLLECTED IN OPEN PONDEROSA PINE FOREST, AT TWO DIFFERENT ELEVATIONS.

SPECIES	NUMBER OF SPECIMENS COLLECTED					
	June 23		June 28		July 14	
	5800′	6700′	5800′	6700′	5800′	6700′
Amphitornus coloradus	.....	.....	.....	.....	1	.....
Chrysochraon abdominalis	.....	1	.....	.....	3	.....
Gomphocerus clavatus	5	6	2	5	1	.....
Arphia conspersa	.....	2	.....	3	.....	.....
Camnula pellucida	.....	.....	.....	.....	.....	2
Xanthippus corallipes leprosus	.....	2	.....	1	.....	.....
Trachyrhachis kiowa kiowa	.....	.....	.....	.....	2	.....
Circotettix rabula rabula	.....	.....	.....	.....	3	12
Hesperotettix viridis viridis	.....	.....	.....	.....	8	.....
Melanoplus dodgei incultus	5	6	.....	6	3	8
M. bivittatus	.....	.....	.....	1	.....	1
M. infantilis	.....	.....	.....	.....	.....	1
M. occidentalis occidentalis	.....	1	.....	.....	.....	.....
M. fasciatus	.....	.....	.....	.....	.....	1
M. mexicanus mexicanus	1	.....	.....	3	2	4

A few species collected are at (or near) their lower altitudinal limit at 6,700 feet. Even early collecting fails to yield many at the 5,800 feet level. Species in this group are *Camnula pellucida*, *Melanoplus dodgei incultus*, *M. infantilis*, and *M. fasciatus*. (The single specimen of the latter was taken at an unusually low elevation for this species in Colorado.)

The role of biotic factors in determining this distribution remains to be determined; e. g., the plant communities in which

[2] *Melanoplus dawsoni* becomes much more common later in the summer than is indicated by the capture of but one specimen. In August it is quite characteristic of the foothills in this part of Colorado, and at that time it occurs in considerable numbers.

collections were made may not be so nearly identical as they seem. Grasshoppers in general are not monophagous, however, and it seems likely that certain climatic factors are of major importance. A final analysis will depend on controlled laboratory experiments, such as have already been conducted by several workers. A certain number of suggestions may, however, be derived from field observations by contrasting

TABLE III.

TOTAL COLLECTIONS, SUMMARIZED.

Species of Acrididae	NUMBER OF SPECIMENS COLLECTED					
	Short Grass		Open Forest		Total	
	5800′	6700′	5800′	6700′	5800′	6700′
Amphitornus coloradus (Thos.)	14	1	1	.....	15	1
Eritettix tricarinatus (Thos.)	8	1	.....	.....	8	1
Chrysochraon abdominalis Thos	2	.....	3	1	5	1
Gomphocerus clavatus Thos	61	8	8	11	69	19
Ageneotettix deorum (Scudder)	9	10	.....	.....	9	10
Arphia conspersa Scudder	1	8	.....	5	1	13
Camnula pellucida Scudder	2	46	.....	2	2	48
Xanthippus corallipes leprosus Saussure[3]	.....	2	.....	3	.....	5
Spharagemon collare (Scudder)	2	.....	.....	.....	2	.....
Trachyrhachis kiowa kiowa (Thos.)	17	7	2	.....	19	7
Circotettix rabula rabula Rehn & Hebard	.....	5	3	12	3	17
Hesperotettix viridis viridis (Thos.)	53	.....	8	.....	61	.....
Melanoplus dodgei incultus Scudder	2	1	8	20	10	21
M. bivittatus (Say)	5	2	.....	2	5	4
M. dawsoni (Scudder)	1	.....	.....	.....	1	.....
M. confusus Scudder	3	.....	.....	.....	3	.....
M. infantilis Scudder	.....	27	.....	1	.....	28
M. occidentalis occidentalis (Thomas)	3	1	.....	1	3	2
M. fasciatus (F. Walker)	.....	.....	.....	1	.....	1
M. mexicanus mexicanus (Saussure)	74	12	3	7	77	19

altitudinal with latitudinal distribution. Moisture, for example, may be important in some cases, but it probably plays a relatively insignificant role in determining the distribution of *Arphia conspersa* or *Gomphocerus clavatus*. These forms have a wide vertical range, which means (in this part of Colorado) that they tolerate a wide range in moisture content of soil and

[3]This should read in all tables *Xanthippus corallipes altivolus* Scudder. I am indebted to Mr. James A. G. Rehn for this correction.

air. On the other hand, they may be very dependent on temperature, for the temperature may be the same at different elevations if measured at the season these forms appear. Length of day is another factor eliminated in these particular cases by the nature of the field observations, the length of day not being a function of the altitude.

The present report is suggestive of the probable results of intensive studies on altitudinal distribution throughout the entire season. Such studies are now being carried out, these including regular collecting at six different altitudes between 5,000 and 10,000 feet, from early spring to late autumn. It is expected that several interesting correlations between seasonal appearance and altitude will be brought to light, and that these, as interpreted in connection with facts already known, may suggest underlying causes of the observed distribution of Orthoptera in the Rocky Mountain region.

MICROSCOPIC OBSERVATIONS ON BLOOD COAGULATION IN SEVERAL DIFFERENT SPECIES OF INSECTS.

J. Franklin Yeager and Harry H. Knight,
Department of Zoology and Entomology, Iowa State College.

It has been shown elsewhere (Yeager, Shull and Farrar, 1932) that blood coagulation in the cockroach, *Periplaneta orientalis*, consists of the formation of a coagulum by the blood cells, the plasma not undergoing obvious coagulation. It was also pointed out that this type of blood coagulation is similar to the blood coagulation of the arachnid *Limulus*, as reported by L. Loeb (1904), and that it seems to fall within one of the three types of crustacean blood coagulation described by Tait (1910–13). The question arises, therefore, is this coagulation picture (marked cell coagulation unaccompanied by obvious plasma coagulation) peculiar to the roach or, on the contrary, typical of insects generally? In an attempt to answer this question the bloods of a number of insects were observed microscopically during the coagulation process. The present paper is a report of the results of these observations.

Material.—The insects used in these studies were obtained[1] at random and used as soon as possible after capture in order to minimize possible subsequent changes in the blood. The bloods of 47 different species were observed, several different individuals and several different drops of blood from each individual being utilized when possible. The animals, with the exception of the field cricket, were collected in spring and summer; crickets were also obtained in the fall.

Method.—The method of microscopic observation is the same as that described elsewhere (Yeager, Shull and Farrar, 1932). A drop of insect blood is submerged in a larger drop of oil (Nujol) on a glass slide and observed without the use of a coverslip. The visible changes accompanying coagulation are

[1]The authors are indebted to various members of the Zoology and Entomology Department (Mr. B. V. Travis, Dr. O. W. Park, Dr. G. C. Decker, Mr. W. R. Grant, Dr. C. J. Drake, Mr. O. E. Tauber, Mr. H. E. Guthrie, Mr. G. L. Weber and Dr. C. H. Richardson) for a number of the insects studied. The authors are further indebted to Dr. F. L. Campbell, of the U. S. D. A., Washington, D. C., for supplying the silkworm larvae and to Mr. C. Wakeland of the University of Idaho, for supplying the Mormon crickets.

noted and the coagulation picture thereby determined. Observation is facilitated by agitating the drop of blood with the point of a needle. In the work reported here, observations were continued until the coagulation process (if any) seemed to have reached completion, the actual observation time varying from animal to animal and ranging from about 20 to more than 60 minutes. Due to the lack of a satisfactory endpoint, no attempt was made to measure coagulation times. All observations were made at room temperature.

Considerable difficulty was experienced in obtaining blood from the aphids. It was found necessary to completely submerge the entire animal in oil on a slide, to then sever one or more appendages with a small scalpel and to then apply enough pressure to the animal's body to force body fluid (blood) from the wounds. The blood thus obtained differed from that obtained from the larger insects in that the latter blood came in contact with air before immersion in oil, while the aphid blood did not have contact with air previous to observation.

Unsuccessful attempts were made to obtain blood from the following insects: *Lecanium corni* (adult), *Gossyparia spuria* (adult), *Aspidiotus ancylus* (adult?), the adult mud wasp, the mole cricket (nymph and adult). Blood was obtained from the honey bee pupa, but the great number of large droplets or spherules which it contained rendered observations unsatisfactory.

Results.—The results obtained are presented in abbreviated form in Table I. The animals are arranged in three groups. *Group 1* includes insects that exhibited no plasma coagulation and no or only a negligible cell coagulation; the insects of *Group 2* exhibited cell coagulation and no or only a negligible plasma coagulation; while *Group 3* includes insects that exhibited plasma coagulation and, usually, some degree of cell coagulation. Without attempting to make quantitative total blood cell counts, the relative numbers of blood cells were roughly estimated as few, moderate and many. The table also indicates the *observed* presence or absence of a granular plasma precipitate in the coagulated blood. The presence of a film at the blood-oil surface was also noted; the appearance of such a film varied considerably from animal to animal, ranging from a thin, colorless film that could be detected only by needle agitation to a conspicuous, relatively thick, granular gel-like membrane.

From the standpoint of blood coagulation, no special significance is here attached to this surface membrane.

It should be emphasized that the three groups, into which the observed insects have been arranged, are not sharply delimited but graduate one into the other. Although most of the coagulation pictures would seem to fall easily within a group, some appear to occur at the border, and their final placement in one group or the other becomes a matter of judgment. The bloods of the potato beetle, *Leptinotarsa decimlineata*, and of *Eucosma otiosana*, for example, exhibited no plasma coagulation and only a slight cell coagulation, while the blood of the walking stick, *Diapheromera femorata*, formed a cell coagulum and what appeared to be a moderate amount of plasma coagulum; the latter animal being arbitrarily placed in group 2. On the other hand, the blood of the water bug, *Belostoma fluminea*, showed a definite plasma coagulation while certain cells cytolysed without the formation of a cell coagulum.

The bloods of the grasshoppers examined seemed to exhibit coagulation of a portion of the plasma. Considerable variation was noted in the quantities of coagulum (both cell and plasma) formed in the cases of both the grasshopper and the May beetle (group 3).

Recognizing, therefore, the intergradation of this grouping, one insect considered illustrative of each group may be selected and the observations of its blood coagulation described.

Group 1.—The honey bee larva[2] seems typical of this group. A drop of blood, having in this case a slightly milky appearance, was obtained from the punctured body wall and placed in oil on a slide. Few cells were present and appeared to be mostly of one or two types, mostly round with fine, almost invisible thread pseudopodia. These cells showed only a slight tendency to agglutinate. Rapid removal of blood from larva into oil followed by immediate observation indicated that some of the blood cells had initial fusiform shapes but quickly lost these by rounding up and assuming circular outlines. The visibility of the cells was somewhat lessened by the slight milkiness or turbidity of the plasma; although no granules were obvious, it is not improbable that the plasma contained a fine granular

[2]Failure of the blood of the honey bee larva to coagulate has been previously reported by Bishop, Briggs and Ronsoni (1925), whose finding is supported by the observations herein recorded.

precipitate. Subsequent agitation of the blood with a needle indicated that no appreciable cell coagulum had been formed and that the plasma had not coagulated, appreciably increased in viscosity or undergone other visible changes. No obvious granular precipitate occurred and no membrane was detectable at the blood-oil surface.

Group 2.—The coagulation picture of the cockroach, *P. orientalis*, is illustrative of this group. Since the coagulation of this insect's blood has been described elsewhere (Yeager, Shull and Farrar, 1932) in some detail, a brief restatement here will suffice: the blood cells tended to round up, became more refractive, developed thread pseudopodia, became sticky, agglutinated and apparently disintegrated, forming a conspicuous cell coagulum. When mechanically agitated the cells became distorted, tending to form long bands of coagulum. This coagulum is referred to as "cell coagulum" and its formation as "cell coagulation," although it is not known to what extent the plasma constituents contribute towards its formation. The main mass of plasma, on the other hand, appeared to form no coagulum and to undergo no obvious increase in viscosity. There usually appeared in the plasma a granular precipitate that seemed to occur initially in the immediate vicinity of the masses of cell coagulum (agglutinated, disintegrated cells). The granules of this precipitate remained in active Brownian movement and showed no tendency to form a fibrous coagulum.

Group 3.—The field cricket, *Gryllus assimilis pennsylvanica*, obtained and examined in the spring and summer, may be used to illustrate this group. A drop of blood taken from a severed antenna and placed in oil rapidly coagulated. The drop contained many cells, of several types. The cells settled to the glass slide surface very slowly or not at all. The yellowish or amber plasma soon became somewhat more deeply pigmented and, at the same time, filled with a heavy granular precipitate, in which the cells appeared to be embedded. This precipitate did not undergo conspicuous Brownian movement as did the plasma precipitate of *P. orientalis* blood; it occupied most of the blood drop and was surrounded by the outer margin of the drop, which consisted of clear fluid with no granular precipitate. Agitation with a needle showed that the large mass of precipitate was associated with coagulated plasma, a fairly sharp line of

demarcation separating the coagulum from the peripheral fluid. This coagulum was sticky to needle, to slide and to itself; it was elastic, apparently contractile and could be removed from the blood drop with the needle point; it was obviously of a fibrous nature, but whether the precipitate constituted the coagulum or whether its granules were merely adherent to an underlying fibrous coagulum could not be determined. In view of the fact, however, that a plasma precipitate may occur in coagulated *P. orientalis* blood, it seems not improbable that the precipitate did not constitute the fibrous coagulum as such.

Discussion.—The observations recorded here were made upon the bloods of insects collected in spring and summer. Since some of the insects observed do not survive the summer or fall and others hibernate in the winter, it is not entirely improbable that the same insect may display a coagulation picture that varies somewhat with the season and stage of metamorphosis or with other factors affecting the physiological conditions of the animal. It should be emphasized, therefore, that the observations listed in Table I are recorded merely as observations and are not to be regarded as generalizations that should hold constant throughout the different seasons of the year or under a variety of environmental or physiological conditions. For present purposes the interest of the recorded observations lies not in the fact that a given species is shown to fall within one or the other of the tentative groups listed in Table I, but rather in the fact that these different types of blood coagulation (and lack of it) can be found in insect blood, especially since these types have been previously recorded by other investigators for crustacean and arachnid blood.[3]

It is of interest, in connection with the remarks just made, that what would seem to be a seasonal variation of the blood coagulation picture has been noted in the field cricket. The observations in Table I and on page 594 for this animal were made with blood taken from individuals collected in the spring after they first appeared. Later observations made in the fall at about the time they were beginning to disappear resulted, in most cases, in a somewhat different picture: the cells formed

[3] In the course of the present work the bloods of *Spirobolus marginatus* (millipede) and of several different species of spiders were observed; in each case, cell coagulation occurred, unaccompanied by plasma coagulation.

a coagulum and, although granules appeared in the plasma, the latter seemed not to form a coagulum, as described above (p. 594) for the blood of this insect. The extent of cell coagulation was also observed to vary considerably in different individuals. It is also of interest, in this connection, that the field crickets caught in the spring were caged indoors without a high mortality while field crickets caught in the fall and retained in cages soon died. Most of the numerous field crickets collected (at random) in the fall were females. Further observations are necessary to determine whether this modification of blood coagulation in the field cricket is a true seasonal variation and whether similar modifications may occur in other species.

Insofar as the present data can be utilized as an index, it would appear that coagulation picture is not correlated to the recognized taxonomic grouping of the observed insects or to the gross stages of development (i. e., larval, pupal or imaginal stages). The observations indicate, however, that when the blood to be examined contains few cells blood coagulation is apt to be slight or not to occur at all, whereas blood exhibiting conspicuous coagulation is apt to contain a considerable number of cells. The insects in group 1, Table I, for example, were observed to possess relatively few blood cells, while most of those in groups 2 and 3 possessed a moderate number of or many blood cells. Many of the animals with few blood cells were imagos. *Belostoma fluminea*, group 3, formed an interesting exception to the above generalization; a blood drop in oil appeared pale green in color and consisted of a clear fluid plasma containing relatively few cells that showed no immediate coagulation changes. Within a few minutes, however, a yellowish granular precipitate appeared in the plasma and seemed to have developed most intensely in the immediate environment of certain cells, cytolysis of which seemed to be coincident with the appearance of the surrounding granules. The yellowish granular mass was slightly sticky to the needle and, upon agitation, became pulled out into strands, giving the appearance of a plasma coagulum. Although the conclusion was reached that coagulation in this animal consisted of cytolysis accompanied by plasma coagulation, without the formation of a cell coagulum, the insect was tentatively listed in group 3, Table I. It is not improbable, however, that this may represent still another type of coagulation, in which case it is of

interest to note that all of the types of crustacean blood coagulation described by Tait (1910–13), L. Loeb (1904), and others, can be found also in insect blood. In general, the blood cells in group 3, Table I, did not appear to agglutinate to a great extent or to form conspicuous cell coagula; it was not determined whether this was due to a natural tendency of these cells to form slight coagula or to an interference with cell agglutination on the part of the coagulating plasma. It is quite probable that an increasing plasma viscosity would tend to inhibit cell agglutination. The plasma coagulum is, in general, surrounded by a clear, uncoagulated fluid, the nature of which has not been determined in this study. It may be "serum" that has become squeezed from the contracting plasma coagulum, somewhat as in the case of mammalian blood. If one assumes, however, that in the insect, as in certain crustacea (see Halliburton, 1885), plasma coagulation is initiated by some substance formed or liberated by coagulated or cytolysed blood cells, it is possible that the clear fluid surrounding the plasma clot may consist of plasma that has not been made to clot. In view of the fact that the plasma coagula observed in the bloods of *Diapheromera femorata* and the adult sphinx moth did not involve the entire plasma, it would appear that the former is the more probable alternative.

Although the present observations offer no basis for estimating the significance of the plasma precipitate, it may be noted from Table I that in non-coagulating blood (group 1) the occurrence of the plasma precipitate is questionable while, in blood exhibiting plasma coagulation (group 3), a conspicuous precipitate occurs; in group 2, characterized by negligible plasma and conspicuous cell coagulation, an obvious plasma precipitate occurs or is lacking in a given insect. From this it would seem that the formation of a conspicuous plasma precipitate is not a necessary accompaniment to the process of cell coagulation in insect blood; the possibility remaining that a microscopically undetected precipitate may have been formed in the plasma of the bloods for which "none" is recorded in Table I.

TABLE I.

RESULTS OF OBSERVATIONS ON BLOOD COAGULATION IN A NUMBER OF DIFFERENT INSECTS.

Group	Order	Species No.	Species Name	Devel. Stage	Relative Number Cells	Cell Coagulation	Plasma Coagulation	Plasma Precipitate[1]	Blood-Oil Membrane	No. Animals Obs.	Average Time Obs. (mins.)	Blood Sample From
1	Homoptera	76	Notonecta sp.; backswimmer	A	Few	None	None	Slight?	None?	2	30	Oar-like leg
		1	Myzus persicae; aphid	A	Few	None	None	None	None	4	30	Appendages[2]
		3	Aphis maidis; aphid	A	Few	None	None	None	None	5	30	Appendages[2]
	Coleoptera	74′	Labidomera clavicollis; beetle	A	Few	None	None	None	None	5	30	Antenna
		75	Tetraopus tetraopthalmus; longhorn beetle	A	Few	None	None	None	Occurs	2	30	Leg
		87	Saperda sp?; longhorn beetle	L	Moderate	None	None	None	Occurs	1	30	Mid-dorsal line
	Lepidoptera	10	Samia cecropia; moth	P	Few	None (aggl.)	None	None	Occurs	2	30	Mid-dorsal line.[3] Wing case
	Hymenoptera	64	Camponotus herculeanus, subsp. pennsyl.; ant	A	Few	None	None	None	None	5	40	Abdom. body wall Thorax, body wall
		34	Camponotus sp?; ant	A	Few	None	None	None	Occurs	1	20	Antenna
		36	Apis mellifica; honey bee	L	Few	None	None	None	None	4	30	Body wall.
		38	Apis mellifica; honey bee	A	Few	None	None	None	None	5	30	Antenna
		78	Hymenopterous parasite[10]	L	Few	None	None	Slight?	None	1	30	Body wall
2	Orthoptera	74	Anabrus simplex; Mormon cricket	N-A	Many	Occurs	None	None	None	5	30	Antenna
		44	Udeopsylla robusta; camel cricket	A	Many	Occurs	None	Occurs	None	4	20	Antenna
		42	Melanoplus differentialis; grasshopper[8]	N-A	Mod. to many	Occurs	None	Occurs	None	9	40	Neck; antenna
		80	Scudderia sp?; katydid	N	Moderate	Occurs	None	Occurs	None	1	30	Leg; antenna
		83	Diapheromera femorata; walking-stick	A	Many	Occurs	None[7]	Occurs	None	1	35	Antenna

See page 600 for explanation of the reference marks.

TABLE I—Continued.

Group	Order	Species No.	Species Name	Devel. Stage	Relative Number Cells	Cell Coagulation	Plasma Coagulation	Plasma Precipitate[1]	Blood-Oil Membrane	No. Animals Obs.	Average Time Obs. (mins.)	Blood Sample From
2	Homoptera	73	Tibicen sp?; cicada	A	Many	Occurs	None	None	Occurs	1	25	Mid-dorsal line
		4	Aphis rumicis; aphid	A?	Few	Slight?	None	None	None	3	30	Appendages[2]
	Coleoptera	7	Carabidae; ground beetle[4]	L	Moderate	Occurs	None	Occurs	None	1	45	Leg
		29	Calasoma calidum Fab.; ground beetle	A	Moderate	Occurs	None	Occurs	Occurs	4	50	Antenna
		17	Elateridae; wireworm	L	Many	Occurs	None	Occurs	Occurs	1	30	Leg
		24	Alobates pennsylvanica, Tenebrionidae; beetle	A	Many	Occurs	None	Occurs	Occurs	4	40	Mid-dorsal neck
		66	Phyllophaga sp?; May beetle	L	Moderate	Occurs	None	None	Occurs	1	40	Leg
		67	Phyllophaga sp?; May beetle	L	Many	Occurs	None	None	None	1	60	Leg
		81	Geotrupes splendidus; scarabid beetle	A	Few	Occurs	None	None	None	1	20	Antenna
		69	Leptinotarsa decimlineata; potato beetle	A	Few	Slight (aggl.)	None	None	None	1	30	Leg
		26	Anisodactylus verticalis Lec.; carabid beetle	A	Few	Occurs	None	None	Occurs	1	30	Antenna
	Lepidoptera	70	Lepidoptera sp?	L	Moderate	Occurs	None	None	None	2	30	Mid-dorsal line
		8	Eucosma otiosana[3]	L	Moderate	Slight (aggl.)	None	None	None	1	40	Body wall
		33	Geometridae; measuring worm	L	Many	Occurs	None	None	None	11	30	Leg
		27	Noctuidae; moth	A	Few	Occurs(?)[5]	None	None (?)	None	1	30	Antenna
		23	Nephelodes emmedonis; cutworm	L	Many	Occurs	None	None	None	1	20	Leg
		86	Sphinx moth	A	Moderate	Occurs	None[7]	Occurs	None	1	30	Mid-dorsal line
		79	Samia cecropia; moth	L	Many	Occurs	None	None	Occurs	2	55	Leg
		82	Bombyx mori; silkworm	L	Many	Occurs	None	Occurs	Occurs	4	60	Leg
		54	Hesperidae; skipper[4]	L	Moderate	Occurs	None	None	Occurs	1	30	Body wall

TABLE I—Continued.

Group	Order	Species No.	Species Name	Devel. Stage	Relative Number Cells	Cell Coagulation	Plasma Coagulation	Plasma Precipitate[1]	Blood-Oil Member	No. Animals Obs.	Average Time Obs. (mins.)	Blood Sample From
2	Diptera	20	Chironomidae; midge	L	Many	Occurs	None	None	None	1	30	Body wall
	Hymenoptera	28	Pteronidea ribesii; sawfly	L	Many	Occurs	None	Occurs	Occurs	14	30	Leg
3	Hemiptera	73′	Belostoma fluminea; water bug	N	Few	Occurs (? cytolysis)	Occurs	Occurs	None (?)	5	30	Leg
		30	Acanthocephala terminalis; Coreidae	A	Moderate	Occurs	Occurs	Occurs	Occurs	1	35	Antenna
	Orthoptera	25	Gryllus assimilis pennsylvanicus; field cricket[5]	N-A	Many	Occurs	Occurs	Occurs	Occurs	20	50	Antenna
	Coleoptera	19	Cucujus clavipes	L	Many	Occurs	Occurs	Occurs	None (?)	1	30	Leg
		6	Phyllophaga futilis; May beetle[4]	A	Many	Occurs	Occurs	Occurs	Occurs	1	40	Leg
		21	Phyllophaga fusca; May beetle[9]	A	Many	Occurs	Occurs	Occurs	None (?)	3	30	Leg
		55	Phyllophaga rugosa; May beetle[4]	A	Many	Occurs	Occurs	Occurs	None (?)	4	30	Leg
	Lepidoptera	77	Datana sp?; caterpillar	L	Many	Occurs	Occurs	Occurs	Occurs	2	50	Leg (false)

[1]Only conspicuous plasma precipitate noted.
[2]Entire animal immersed in oil (Nujol) and appendages severed with scalpel; see text.
[3]Kept several weeks in ice box at about 5° C. before use.
[4]Dug out of ground in spring.
[5]Though no definite plasma coagulum cound be detected, the plasma appeared to increase somewhat in viscosity.
[6]What appeared to be a cell coagulum was obscured by the presence of dark masses of brownish granules or droplets (spherules?).
[7]Placed in group 2, although a portion of the plasma seemed to form a plasma coagulum.
[8]See text for observed variations in coagulation picture of this insect.
[9]Animal had been flying previous to capture.
[10]Larva found inside dead cicada.

The "cell coagula" of the insects of group 2 seemed to present two different appearances. Some coagula, of which that of *P. orientalis* is an example, consisted of agglutinated cell masses of a quite granular appearance, the individual cells composing the masses of coagulum tending to become lost to view. On the other hand, some coagula, of which that of *Anabrus simplex*, the Mormon cricket, forms an example, consisted of agglutinated cell masses of a relatively non-granular appearance, the cells of the coagulum remaining clearly visible and apparently not becoming obscured by a granular formation. The question arises, does the former type of cell coagulum have its granular appearance due to being associated with a small amount of plasma coagulum or not? Since further work is necessary to answer this question, both types of coagula are tentatively designated cell coagula and are included in group 2; group 3 containing, with the exception of *Belostoma fluminea*, only those animals in which most of the plasma appeared to be involved in the plasma coagulum.

SUMMARY.

1. Microscopic observations have been made upon the bloods of 47 different species of insects and the coagulation process noted.

2. The method used consists of submerging a drop of insect blood in a larger drop of oil (Nujol) on a clean glass slide, observing microscopically and, when desirable, agitating with the point of a needle.

3. The results are condensed and recorded in Table I, in which the animals are arranged into three tentative groups according to the coagulation process observed. *Group 1:* No plasma coagulation and no or only a negligible cell coagulation; *Group 2:* Cell coagulation and no or only a negligible (slight?) plasma coagulation; *Group 3:* Marked plasma coagulation and, usually, some degree of cell coagulation. One insect included in group 3 showed cytolysis, plasma coagulation and no cell coagulum formation; further observations may indicate this represents a fourth group.

4. An apparent seasonal variation has been noted in the blood coagulation of the field cricket, *Gryllus assimilis pennsylvanica;* it was observed that plasma coagulation occurred

to a marked degree in the spring and to only a slight degree in the fall (1932).

5. Within the species examined, the blood coagulation bears little or no relation to recognized taxonomic grouping or to gross stages of development (for example, all larvae do not show the same coagulation changes).

6. The types of blood coagulation observed and recorded here conform to the types already recognized as occurring in crustacean and arachnid (*Limulus*) blood. (See Tait, 1910–13.)

7. It would seem that the appearance of a conspicuous plasma precipitate is not a necessary accompaniment of cell coagulation in the insect.

8. The plasma coagula recorded in group 3, Table I, were surrounded by a peripheral layer of clear fluid, the nature of which has not been determined.

9. Granular and non-granular cell coagula (group 2) of two different appearances were noted.

10. The grouping of species in Table I is tentative and may require modifications based upon more extensive observational and experimental investigation.

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EFFECTS OF ULTRA VIOLET RADIATIONS ON THE BEAN WEEVIL, BRUCHUS OBTECTUS SAY.

G. F. MacLeod,
Ithaca, N. Y.

The rapid progress made during recent years in the study of light as a physical phenomenon has suggested the desirability of further investigations in the field of optics with reference to insects. A review of the results obtained from previous studies of ultra violet light as a factor in physiological processes indicates that the chemically active rays of shorter wave length have a pronounced effect upon the general functions of any organism. This effect has been revealed in modifications of both metabolism and movement. The bactericidal action of ultra violet light has been substantiated by numerous experiments. Research in the field of medicine amply demonstrated the injurious effects of prolonged exposure to ultra violet irradiations both to the skin and eyes of humans. Seide (4) found the females of *Hydatina senta* were killed after irradiation for five minutes with ultra violet and 70 percent of the eggs carried by irradiated females were injured. Geigy (2) reports abnormalities in *Drosophila* caused by treatment with ultra violet light. Prolonged exposure to radiant energy of shorter wave length may be detrimental to many kinds of animals, and some of the general effects of this energy upon the common bean weevil are reported in the present work.

METHODS.

The equipment used in all the following experiments was manufactured by the Hanovia Chemical Co. under the trade name of "Home Model Alpine Sun Lamp." The effective luminous part of the burner, a Cooper-Hewitt product, consisted of two parallel arms each about two inches in length and one-half inch in diameter. A transformer and rectifier was provided for operation on a 100-volt, 60-cycle alternating current. The arc started with a current of 5.5 amperes and ran at 3.5 amperes. About 35 minutes was required for the outfit to reach maximum intensity and electrical equilibrium. A careful check of the power input during radiations showed only slight variations. Both transformer and burner were new, but the lamp was operated for about 50 hours before any irradiations were attempted. All of the

experiments were performed before the burner had reached an operating age of 300 hours. Before starting each experiment the burner was carefully cleaned, the arc struck and an equilibrating period of 35 minutes elapsed before specimens were exposed. All distances indicated were measured directly in front of the lamp from the seal-off tip of the burner. Exposures were made parallel to the arc in every case.

Energy distribution curves and a complete discussion of the quartz-mercury arc have been published by Anderson (1). Further measurements of spectral energy values (Table I) have been furnished by Dr. Ralph A. Sawyer, of the Department of Physics at the University of Michigan.

TABLE I.

ENERGY VALUES OF LINES IN THE ULTRA VIOLET SPECTRUM.

Wave Lengths Å Units	Watts/mm²	Wave Lengths Å Units	Watts/mm²
2399	6.0×10^{-8}	3022	33.8×10^{-8}
2483	11.3×10^{-8}	3125-32	77.9×10^{-8}
2536	38.5×10^{-8}	3342	10.0×10^{-8}
2652	23.8×10^{-8}	3473	3.1×10^{-8}
2753	6.0×10^{-8}	3650-54	111.4×10^{-8}
2804	12.8×10^{-8}	3790	4.2×10^{-8}
2894	7.1×10^{-8}	4047	49.4×10^{-8}
2967	17.7×10^{-8}	4339-58	73.0×10^{-8}

For the purpose of eliminating wave lengths shorter than 3,000 Angstrom units a piece of plain glass 2.5 millimeters in thickness was employed as a filter. The transmission accomplished by this arrangement is shown in Fig. 1, Plate I, and Text Fig. 2. The data presented were taken at the close of all experimental work. In all of the present work "Radiation A" refers to the full transmission of the arc, while "Radiation G" refers to the transmission obtained by using the glass screen with the arc.

EXPERIMENTS WITH EGGS.

In the first test with the mercury arc 300 eggs of known age were secured from the same individuals. These eggs were irradiated at a distance of 30 cm. from the light. The duration of treatment was 15 minutes in which period the maximum temperature attained was 27° C. The eggs were then incubated

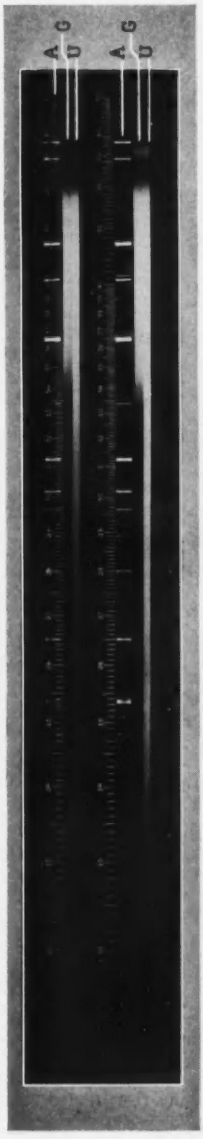

FIGURE I

SPECTRAL TRANSMISSION OF QUARTZ-MERCURY ARC AND GLASS SCREEN

A–The line spectrum of the quartz-mercury arc

G–The spectral transmission of the glass screen

U–The continuous spectrum of an under-water spark

at 32° C. in a constant temperature oven. Suitable controls were kept under identical conditions except for exposure to light from which they were shielded by an opaque paper screen.

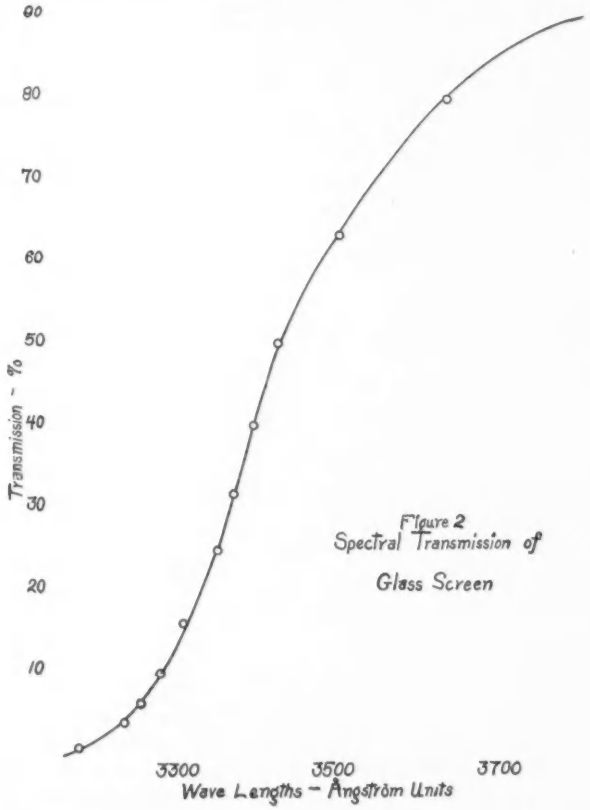

Figure 2
Spectral Transmission of Glass Screen

As indicated in Table II, there was a considerable variation in fertility of the eggs, but the destructive effects of the arc were apparent in every case. Eggs irradiated five days after deposition collapsed just prior to larval emergence, while eggs 24 hours old collapsed shortly after treatment without further embryonic development. The lethal effects of the light seemed to vary inversely with the age of the eggs.

TABLE II.

EFFECTS OF IRRADIATING EGGS WITH RADIATION A.

TREATMENT	AGE OF EGGS WHEN IRRADIATED (days)	HATCHING (Percent)	INCUBATION PERIOD		
			min. (days)	av. (days)	max. (days)
Irradiated	1	43.3	6	6	6
Control	1	97.1	5	6	6
Irradiated	3	0	0	0	0
Control	3	9.0	7	7	7
Irradiated	5	14.2	8	10	11
Control	5	56.0	8	8	9
Averages Irradiated		6.16	7	8	8.5
Averages Control		54.03	7	7	7

TABLE III.

EFFECTS OF DURATION OF IRRADIATION ON EGGS OF THE BEAN WEEVIL.

TIME OF EXPOSURE MINUTES	PERCENTAGES OF EGGS		FEEDING SPOTS ON BEANS TOTAL NO.	TOTAL ADULT EMERGENCE PERCENT
	Hatched	Collapsed		
5	26.5	73.5	15	6
10	20.0	80.0	5	0
15	26.5	73.5	6	0
20	6.7	93.3	7	0
25	13.3	86.7	5	0
30	0	100.0	0	0
40	0	100.0	0	0
50	6.7	93.3	1	0
60	0	100.0	0	0
Controls	93.0	7.0	–	67

A group of newly-emerged adults were isolated and a supply of eggs of known age was secured. The eggs were placed on flat black paper and exposed for from 5 to 60 minutes at a distance of 35 centimeters to all rays of the mercury arc. At the end of each five minutes of treatment 45 eggs were selected from the group and placed 15 in a vial with 10 kidney beans. Parallel control forms were similarly handled, but shielded from the light. The maximum temperature attained during radiation was 25° C., which was well within the limits permitting normal development. Upon completion of the treatment, all of the containers were placed in the 32° C. oven at

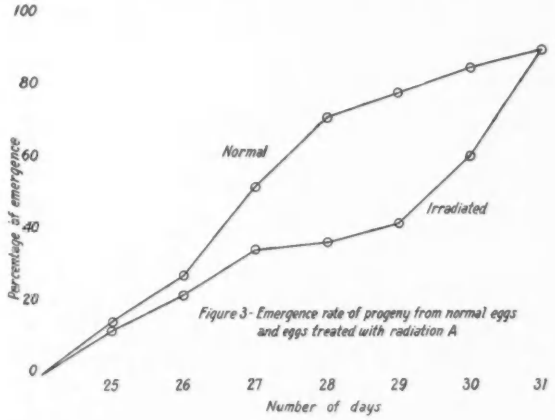

Figure 3- Emergence rate of progeny from normal eggs and eggs treated with radiation A

approximately 87 percent relative humidity. The vials were examined daily and observations as to development recorded.

There can be little doubt as to the lethal effects of light from a quartz-mercury arc on eggs of the bean weevil (Table III). Dosages for as short a period as five minutes reduced the viability of eggs although not all of them were killed. The different positions which eggs may assume with respect to direction of the light rays may possibly explain the variations encountered in these tests. Exposures longer than 15 minutes in duration killed a majority of the eggs, while with the few which did hatch no subsequent growth occurred. Numbers of feeding spots on beans were of significance in that these areas gave a rough indication of the activity of larvae which

hatched from irradiated eggs. In all of the treatments there were many dead larvae which were unable to enter beans although in some cases, several attempts were made, as was evident from the feeding. Of the collapsed eggs the majority showed embryos which were nearly fully developed within the chorions. No structural abnormalities were observed in larvae

TABLE IV.

EFFECT OF RADIATION G ON EGGS.

PERIOD OF EXPOSURE	EMERGENCE TOTAL	INJURED BEANS	ADULTS PER BEAN	AVERAGE ADULT WEIGHT	FOOD CONSUMED	
					PER ADULT	PER MG. OF WEIGHT
minutes	percent	percent	av. no.	mgs.	mgs.	mgs.
5	81.3	60	2.6	6.4	7.5	1.18
10	100.0	60	3.3	6.1	6.5	1.07
15	91.4	60	3.0	5.6	7.4	1.32
20	89.6	40	4.5	6.0	9.9	1.65
25	91.0	80	2.2	5.8	6.9	1.19
30	50.5	40	2.5	6.1	9.7	1.59
35	81.1	60	2.6	6.2	8.3	1.34
40	82.0	60	2.6	6.1	8.7	1.43
45	27.0	40	1.0	6.6	12.9	1.96
50	78.1	60	2.3	6.6	8.5	1.28
55	70.4	60	2.3	6.9	10.4	1.50
Averages	76.9	56.6	2.6	6.3	8.88	1.40
Controls	86.7	60	4.3	5.7	6.5	1.14

hatching from treated eggs which would account for their inability to enter beans.

The eggs used in tests with radiation G were obtained from normal beetles and were from 24 to 72 hours old at the time of irradiation. Exposure periods ranged from 5 to 60 minutes at a distance of 30 centimeters from the arc, during which time the temperature was 25 ± 1° C. A total of 720 eggs were involved in studies, the results of which are presented in Table IV.

Radiation G produced no marked deviations from normal development in progeny from irradiated eggs. In contrast to the destructive influence of shorter wave lengths, radiations longer than 3,126 A may possibly have had a slight stimulative effect, as is indicated by the greater average weight of treated forms. An examination of the total moisture in irradiated and control beetles gave 44.5 percent in case of the former and 47.3 percent with the latter.

A comparison of emergence curves of normal and treated individuals in these experiments fails to disclose any significant

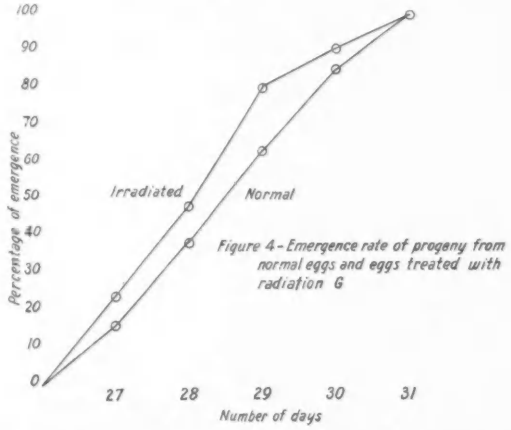

Figure 4 - Emergence rate of progeny from normal eggs and eggs treated with radiation G

differences as is shown in Figure 4. Adult emergence was comparatively uniform in this series of tests and was completed in relatively fewer days than is ordinarily the case even with normal insects. The similarity of progeny from control and exposed forms suggested an absence of any destructive effects from radiation G, and at the same time indicated those wave lengths shorter than 3,126 Å as the probable causative agents in defective metabolism or death from exposure of eggs to a quartz-mercury arc.

EXPERIMENTS WITH LARVAE.

Because previous experiments had demonstrated that tests started with normal larvae resulted in a high mortality, probably because of faulty technique in handling, irradiation experiments

were attempted with these forms. A group of 200 newly emerged specimens from normal eggs of the same individuals was divided into two equal lots. Half of these were irradiated for five minutes at a distance of 30 cm. while the remainder were kept as controls. Both controls and treated forms were subjected to temperatures which at no time exceeded 30° C.

None of the irradiated larvae were able to effect an entrance to the beans, although they were alive when placed in the vials.

TABLE V.

RESULTS OF IRRADIATING ADULTS FOR VARYING PERIODS OF TIME.

TIME OF EXPOSURE	PERCENTAGES OF EGGS		FEEDING SPOTS ON BEANS	TOTAL ADULT EMERGENCE
	Hatched	Collapsed		
Minutes 10	Percent 100.0	Percent 0	Total Number 13	Percent 0
20	20.0	80	6	0
30	26.5	73.5	4	0
40	13.3	86.7	0	0
50	26.5	73.5	2	0
60	20.0	80.0	1	0
Controls	100.0	0.0	...	86.7

Of the control larvae 14 emerged as adults, only about half of which were normal in size. This was due in part at least to the fact that only two beans were entered and consequently the food supply was deficient. The average weight of emerging adults was 3.88 mgs. They consumed 22.95 mgs. of food per adult, which was at the rate of 5.91 mgs. per milligram of body weight. While these control forms were below normal, the fact that no sign of life was evident with irradiated larvae indicated an injurious action of ultra violet light.

EXPERIMENTS WITH ADULTS.

Exposures of adults to ultra violet light produced no significant changes in the beetles, but variations from normal were observed in the progeny of treated forms. To obtain

further information on this subject a number of newly emerged beetles from the stock supply were irradiated. The fertility of eggs produced was very low in all cases of treated adults. The experiment was repeated three times in an attempt to obtain a sufficient number of eggs upon which to base conclusions. Vials with ten eggs and ten beans representing the various periods of treatment together with suitable controls were kept in the 32° C. oven until the experiment was completed.

Decided effects of the light upon the reproductive powers of adult weevils are apparent from the results expressed in Table V.

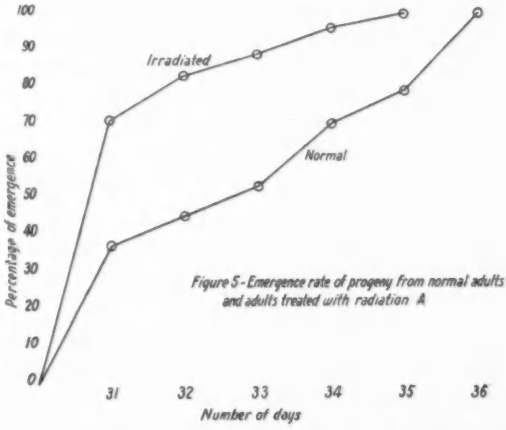

Figure 5-Emergence rate of progeny from normal adults and adults treated with radiation A

Exposure of adults for 20 minutes or longer materially reduced the viability of eggs produced. With 10-minute treatments embryonic development was not inhibited, but none of the larvae which hatched were able to enter beans. No attempt was made to orient adults with respect to direction or points of incidence of the light rays. Due to phototropic reactions the heads and ventral surfaces of the abdomens received the major part of light. There seemed to be no obvious connection between regions of the body which were exposed and the general effects upon reproductive powers of treated insects.

A consideration of emergence rate of progeny from beetles irradiated with dosages of less than 10 minutes duration (Figure 5) discloses a reversal of conditions as found with

progeny from irradiated eggs. The time of emergence was delayed by exposing eggs to the light rays and accelerated when adults were treated. The pupal stage was three or four days longer than usual in the case of all treated forms. This is of particular significance in considering abnormalities in specimens originating from irradiated adults.

In general there were no lethal effects of mercury arc radiations as far as the adults themselves were concerned. The length of adult life was not noticeably shortened, but the effects of irradiation were apparent in progeny of treated beetles. Exposure of adults for 20 minutes or longer at a distance of 30 centimeters reduced the viability of eggs produced. A further effect was evident in that those forms hatching from eggs deposited by irradiated beetles were unable to enter beans. At distances of from 30 to 90 centimeters from the arc no effects attributable to varying intensities of radiations were discernible, but all irradiated material exhibited metabolic disturbances. Larvae from eggs of treated adults consumed more food than normal specimens, but failed to build correspondingly greater adult weights.

ABNORMALITIES.

The activity of x-rays in producing abnormal morphological developments in insects is well known. A study of external abnormalities in development of *B. obtectus* produced or accentuated by exposure to light of ultra violet wave lengths indicated certain deformities which are of interest. Four generations of adults were secured, starting with irradiated eggs in one case and irradiated adults in another group. The structures observed represent results of a single treatment and not cumulative effects of several radiations. Larvae of the first generation from irradiated adults were frequently unable to complete pupal processes and the resulting forms were admixtures of larvae, pupae and adults. These deformities occurred only in the first generation and were probably associated with the disturbances of metabolic processes already noted. Progeny from both irradiated adults and eggs had noticeably shortened wing covers or swollen abdomens. In the third and fourth generations of beetles from irradiated forms there was a much higher proportion of individuals presenting this abnormality than was the case with preceding

generations. The effects of exposure to short wave lengths of light as indicated in external abnormalities seemed to be firstly a direct physiological disturbance of the forms exposed, and secondly an influence expressed as a structural variant which may be transmitted genetically from one generation to another.

SUMMARY.

Eggs and first instar larvae of the bean weevil were killed by exposure to wave lengths of light shorter than 3,126 Å from a quartz-mercury arc. Adults exposed to these same rays exhibited no marked effects, but eggs from these forms were largely sterile. Sublethal dosages produced forms which were defective in their metabolic processes as was evidenced by the excessive amount of food consumed and failure to build a correspondingly greater body weight.

Abnormalities of external structures were apparent in forms from both irradiated eggs and adults. Shortened elytra, or swollen abdomens, an abnormality accentuated in the third and fourth generations, was very common in progeny of all irradiated forms. Larvae developing as progeny of irradiated adults sometimes had a prolonged pupal period with the resulting forms becoming mixtures of larvae, pupae and adults.

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DESCRIPTIONS OF TWO NEW ANTS OF THE GENUS PHEIDOLE.

(Hymenoptera: Formicidae.)

A. C. Cole, Jr.,
Twin Falls, Idaho.

Pheidole pilifera subsp. **artemisia** subsp. nov.

Worker—Length, 2.5–3 mm.

Head, excluding mandibles, longer than broad; coarsely, irregularly and deeply rugose; vertex excized. Frontal area convex, black, smooth, shining and elliptical; apex somewhat pointed and impressed. Clypeus unnotched; frontal carinae pronounced. Mandibles 6-toothed, rugose; apical tooth short and pointed, other teeth short and uneven. Diameter of eyes one-fourth length of genae. Antennal scapes inwardly curved; funiculi half again as long as scapes; last joint of antennal club longer than both basal joints taken together.

Thorax, petiole and post-petiole finely and rather evenly punctate except for a very small area on dorsum of the pronotum, which is smooth and shining. Apex of petiole blunt, slightly rounded, without a median impression. Surface covered with fine, yellowish, erect or suberect hairs; on antennal scapes suberect and short, on funiculi suberect and short but more dense; erect and long on head and gaster; shorter on thorax, petiole and post-petiole; on tarsi of foreleg short, suberect and rather dense, on other legs shorter and more sparse, shorter on flexor surfaces. Tibiae of hind legs more hairy than of fore or middle legs. Pubescence lacking.

Head, thorax, petiole and post-petiole subopaque; legs and gaster shining. Head, antennal scapes, thorax, femora of legs, petiole and post-petiole dark reddish brown; pronotum slightly lighter. Mandibles and antennal funiculi testaceous; club fuscous. Legs, except fore part of tibiae, testaceous; fore part of tibiae reddish brown.

The worker of *artemisia* differs from that of the typical *pilifera* in the following characters: Head smaller, longer and less broad; frontal carinae less pronounced; antennal funiculi decidedly longer than the scapes; last joint of antennal club longer. Smooth and shining area on dorsum of the pronotum; apex of petiole less pointed; post-petiole somewhat shorter and more prominently elevated. Tip of gaster less pointed and more elliptical. Rugosity coarser, deeper and more irregular. Color of body much darker.

Soldier—Length, 3.5–4 mm.

Head, excluding mandibles, longer than broad; vertex deeply and broadly excized. Frontal carinae pronounced; frontal area convex, shining, pointed at apex, angular at base. Genae not greatly convex. Eyes small, about one-seventh length of genae. Occipital angle sharp. Antennal scapes strongly and inwardly curved, two-thirds as long as funiculi, broad and flattened at base and apex, more slender in center. Mandibles strongly convex and smooth, with bases very faintly rugose or punctate; apical tooth large and curved, other teeth small and uneven.

Thorax finely punctate, about twice as long as broad; pronotum angular; mesepinotal constriction pronounced; epinotal spines short and pointed, shorter in proportion to those of worker; petiole as wide as high, broadly but not deeply notched.

Gaster broadly elliptical; smaller than head.

Surface covered with erect and suberect whitish hairs; suberect, short and sparse on antennal scapes; suberect and more dense on funiculi; long, erect and abundant at tip of gaster; head less hairy than that of worker.

Frons of head coarsely and rather unevenly rugose; lower portion of genae finely rugose, upper portion smooth; vertex coarsely and deeply punctate. Petiole and post-petiole finely punctate.

Reddish brown; antennal funiculi and tarsi of legs lighter; vertex and frons of head and the thorax darker; head lighter ventrally; mandibular teeth, petiole, post-petiole and gaster black. Rugose and punctate areas of head infuscated. Post-petiole and gaster shining; remainder of body subopaque dorsally, shining ventrally.

The soldier of *artemisia* differs from that of the typical *pilifera* as follows: Head smaller, longer and less broad; frontal area decidedly more convex; frontal carinae less pronounced; vertex less sharply excized; antennal scapes much shorter than funiculi; mandibles less punctate and pilose; vertex and occipital region much less coarsely punctate. Rugose areas absent on thorax; epinotal spines shorter, straighter and more blunt; apex of petiole more broadly notched, less hairy; post-petiole rather globular, less angular and not as broad. Gaster not greatly smaller than head. Coarser deeper and more irregular rugosity. Color of entire body much darker.

Described from a series of 43 workers and 17 soldiers collected by the writer at Provo, Utah, on a hillslope covered with *Artemisia tridentata* Nutt. The nest was a small chamber beneath a large flat rock. The ants were sluggish and timid.

Cotypes are in the collections of C. H. Kennedy and the author, and topotypes in the collection of M. R. Smith.

Pheidole californica var. **shoshoni** var. nov.

Worker—Differing from the worker of the typical *californica* as follows: Thorax, legs, antennae, clypeus, mandibles and petiole varying from light tan to testaceous; post-petiole, gaster and head, except appendages and clypeus, darkly testaceous; genae slightly lighter; antennal articulations fuscous. Surface rather smooth and shining, head and gaster more so than the thorax; tibiae transversely or longitudinally striate; head and gaster more hairy than thorax.

Soldier—Differing from the soldier of the typical *californica* in the following points: Vertex of head deeply excized. Surface rather evenly and densely hairy, but hairs on petiole and post-petiole long, on legs sparse and on epinotum very sparse or altogether absent. Head, pronotum, and mesonotum shining; gaster more so; epinotum and coxae of middle and hind legs subopaque. Occipital region of head finely reticulate; frons finely and longitudinally rugose.

Legs, petiole and thorax, except pronotum, uniform light tan; pronotum, head, post-petiole and gaster somewhat darker; mandibles a rich brown, their teeth dark brown or black. Anterior portion of gaster lighter than the thorax, posterior portion darker. Rugose areas lightly infuscated.

Described from numerous workers and soldiers collected by the author in the Snake River Canyon, near Twin Falls, Idaho. The nest was beneath a flat rock on the bank of a small stream in an area of *Hordeum jubatum*. Colonies of this variety are abundant in the type locality and are all rather populous.

Cotypes are in the collections of C. H. Kennedy and the author.

NOTES ON SOME SOUTH AMERICAN MOSQUITOES.

II. The genus *Mansonia*, subgenus *Rhynchotaenia*[1-2]

NELSON C. DAVIS, M. D.
From the Yellow Fever Laboratory of the International Health Division of the Rockefeller Foundation, Bahia, Brazil.

It is believed that the later aquatic stages of the mosquito group *Rhynchotaenia*, of the genus *Mansonia*, have never been adequately described. Goeldi (1) has described and figured the ova and first-stage larvae of *Mansonia* (*Rhynchotaenia*) *fasciolata* and of *M.* (*R.*) *arribalzagae*. From time to time during several years various workers at Bahia have attempted to secure larval forms of this subgenus, but until recently they have met with no great success. Some half-dozen larvae were found on the roots of a small sedge, and two or three were taken from the roots of common grasses growing at the edge of a stream; but all of these died without pupating. However, since early in May, 1933, larger collections of larvae have been secured from a tall cane-like sedge of the family Cyperaceae (*Fuirena umbellata* Rottb.)[3] whose roots are buried in mud or slime, usually overlaid with several inches of water. This plant grows abundantly in certain swampy valleys less than five miles from the city of São Salvador. Plate I shows one of these breeding areas; also a close view of one of the plants.

Before larvae had been found in large numbers in nature, it was thought that perhaps they might be reared from eggs. In a previous publication (2) are recorded the results of various captures of adults made in 1932. In the present year (1933) another series of captures has been made with animal bait at Pirajà, near São Salvador. Table I gives the results of these captures. Live females were carefully identified and those of the four species were placed in separate cages. Each cage was provided with a bowl of water containing small sedges, known

[1]The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of the Rockefeller Foundation.

[2]Part I of this series, see Davis, Nelson C., Ann. Ent. Soc. Amer., 26: 277-295, 1933 (June).

[3]For the identification of this plant the writer is indebted to Dr. A. da Costa Lima of the Botanical Garden in Rio de Janeiro.

to harbor, at times, larvae of the group; and with a dish of honey. Three times a week a bound guinea-pig was placed in each cage to furnish blood. Eggs were laid from five to nine days after capture by *M. justamansonia* and by *M. fasciolata;* none were laid by *M. albicosta* or by *M. chrysonotum.*

TABLE I.

Night Captures of *Mansoniae.*

Date	Manner of Capture	Species							
		M. justa-mansonia		*M. fascio-lata*		*M. albi-costa*		*M. chryso-notum*	
		Fe-males	Males	Fe-males	Males	Fe-males	Males	Fe-males	Males
March 2, 1933	Picked off of horse with tubes	23	1	16	.....	9	.....	2	.....
	Caught with net in air above horse	4	16	1	.....	.....	.....	.....	.....
March 8, 1933	With tubes	78	.....	36	.....	37	.....	2	.....
	With net[4]	42	27	5	.....	4	.....	1	.....
March 15, 1933	With tubes	157	3	61	.....	14	.....	4	.....
	With net	98	151	17	.....	3	.....	2	.....
March 24, 1933	With tubes	155	1	17	.....	20	.....	3	.....
	With net[4]	109	140	6	.....	6	.....	1	.....
	Totals	666	339	159	.....	93	.....	15	.....

Egg rafts have always been found on the surface of the water, not on the plants. Rafts invariably consist of two long parallel files of eggs, cemented together. At one or both ends of the raft the eggs stand side by side, but in the course of the rows the positions frequently alternate owing to slight differences in size between individual eggs. (See Plate II, Fig. 3). The color is very dark brown, almost black. The numbers of eggs per raft of *M. justamansonia* found to date are: 88, 98, 57, 69, 80, 116, 72, 140; the numbers for *M. fasciolata* are: 26, 77, 114, 127.

[4]Upon each occasion two mosquitoes were taken which were mutilated beyond recognition.

Microscopically the eggs are thickly studded with minute conical air cells. This appearance is shown semidiagrammatically in Plate II, Fig. 4; actually the air cells are even more thickly set. No differences have been found between the eggs of the two species.

The eggs hatched three to four days after discovery. The first stage larvae of the two species are very similar (Plate II, Figs. 5 to 8). Anal gills and antennal hairs appear to be slightly longer in *M. justamansonia;* length of larvae and length of comb teeth appear to be slightly greater in *M. fasciolata.* The first-stage larvae invariably died in two or three days.

During May, June, and July, 1933, a total of 1367 larvae were taken from the large sedges previously mentioned. In nature only two pupae were collected. It is probable that the pupae become loosened as the plants are pulled up. The larvae seem to thrive in thin mud. Their color is grayish, or faintly yellowish, frequently almost white. A few of them were preserved for mounts; the others produced adults as follows: 155 *M. justamansonia,* 9 *M. fasciolata,* and 5 *M. chrysonotum.* Some of the emergences came from isolations, others from pooled collections of larvae. Isolated larvae were reared on a species of small sedge. The finer rootlets of the preferred plant are partly stripped when pulled from the mud; the coarser roots appear to be not so suitable for attachment. There is no way of knowing the length of the larval stage. From 42 among 45 pupae of *M. justamansonia* for which records are available, adults emerged on the seventh, eighth, or ninth day. Two (both males) emerged before the seventh day, and one (also a male) emerged as late as the eleventh day. The average pupal period was just under eight days (7.8 days) and there was no significant difference between the mean period for males and that for females.

Fourth-Stage Larva of **M. justamansonia** (Chagas).

(Fig. 9.)

Head transverse, frontal spines projecting, anterior border flat or slightly convex. Head hairs tufted, the lateral ones more strongly than the median. Shaft of antenna longer than head; hair tuft at outer third, with 16 to 20 branches, shaft tipped by two spines and a filament which is longer than the shaft. Internal structure of filament resembles a coiled spring; chitinized rib, which appears to have separate origin from shaft, supports filament laterally; filament tipped by two

small spines (Fig. 16). Anterior submedian thoracic group consists of three long hairs, arising close together; this group is flanked by a small tufted hair half-way between mid-line and shoulder of thorax.

Lateral comb of eighth segment consists of 9 to 14 teeth or scales in a single row; teeth decrease in size toward ventral end of row (Fig. 10). Air-tube short and stout, outer two-fifths sharply narrowed and heavily chitinized; tip carries about 12 retrose hooks. At shoulder preceding narrowed tip arise dorsally two curved blade-like setae; these are hinged, and may be found either erect or fitted along dorsal surface of air-tube; in latter position their tips coincide with extremity of tube; upper surface of setae finely serrate (Fig. 18). Probable function of setae is to aid in puncturing roots and in enlarging the openings. Tip of air-tube appears to be formed by two closely approximated plates; these are coarsely serrate along upper surface. In addition to the setae, three pairs of more slender hairs arise near the level of shoulder of air-tube; most ventral pair with hairs bifurcated near base. Distal to comb on lateral surface of eighth segment is a group of three hairs, one long single and two small tufted ones (Fig. 12).

Anal segment twice as long as broad, ringed by the plate; basal fourth and a narrow segment at tip not chitinized; ventral brush posterior; two dorsal tufts (one with about seven, the other with about three elements) on each side; chitinized ring pierced by two pairs (one ventral, one dorsal) of small tufted hairs. Anal gills slender, shorter than segment.

Although larval skins of both *M. fasciolata* and *M. chrysonotum* have been saved, it is impossible from these mounts to make minute comparisons of their pilotaxy with that of the larvae of *M. justamansonia*. In general the larvae of the three species are very similar. Small differences have been found in the antennae (Figs. 15, 16, 19). It will be noted that the tufted shaft hair of *M. justamansonia* has fewer branches than that of *M. chrysonotum* and more than that of *M. fasciolata*. As previously stated, the longest hair in the lateral group posterior to the comb is single in *M. justamansonia;* in the other two species it is bifurcated (Figs. 12, 13, 14). Minor differences exist in the teeth of the comb (Figs. 10, 11, 23); those of *M. chrysonotum* are decidedly long and slender. The species appear to be clearly separable by the shape of their mental plates (Figs. 20, 21, 22). Pupae of the three species resemble one another closely, although minute differences have been noted in the respiratory trumpets. Pupal hairs are all extremely small (Plate VI, pupa of *M. justamansonia*).

Mansonia (Rhynchotaenia) fasciolata (Lynch Arribálzaga).

The writer has specimens of *M. fasciolata* from Bolivia and from six Brazilian states, ranging from São Paulo in the south to Pará in the north. All these mosquitoes differ from the description given by Dyar (3) in that the wings show some white scaling. In this respect they conform more to the descriptions published by Bonne and Bonne-Wepster (4) and by Shannon (5). By Dyar's key these specimens would undoubtedly be identified as *M. venezuelensis*. It is very probable that the latter name is a synonym of *M. fasciolata* (6). There is always present a subbasal white patch on the costa. This white area is very small in the examples from Pará, but in those from Bahia it frequently occupies nearly one-tenth of the distance from base of costa to tip of first vein. Beginning more distally than the costal spot, is a smaller one on the first vein; this is also constantly present. In addition to these two definite aggregations, there are always a few white scales scattered sparsely along the costa, subcosta, and first vein, but never elsewhere.

Although Dyar (3) figures the male genitalia of *M. fasciolata*, his illustration is so small that details are lacking. Plate VII shows drawings of the terminalia of specimens bred out from larvae collected at Pirajá, near São Salvador, Bahia. It should be pointed out that the basal lobe of the side-piece carries two terminal spines which appear to be fused through much of their length, as in *M. justamansonia* (2). Specific differentiation from *M. justamansonia* may be made on the shape of the mesosome and its fewer number of teeth (Figs. 31, 32).

Mansonia (Rhynchotaenia) albicosta (Chagas, in Peryassú, 1908).

Although adults of *M. albicosta* are fairly abundant in the region where the related species are found, none has emerged from the larval collections made to date. Either the larvae attach themselves to some other plant and cannot be found, or they are so delicate that they die in the laboratory at some stage before emergence.

Mansonia (Rhynchotaenia) albifera Prado (7).

From the description of this species and from the figure of the genitalia presented, it is safe to assume that the author was working with *M. chrysonotum* (Peryassú). *M. albifera* becomes, therefore, a synonym of *M. chrysonotum*.

Mansonia (Rhynchotaenia) araozi Shannon and Del Ponte (in Dyar, 1928).

In a collection made near Roboré in western Bolivia, February, 1933, there have been found two imperfect specimens which may be *M. araozi*, although they do not agree in all respects with Shannon and Del Ponte's description. The same collection contained *M. fasciolata*. The mosquitoes were captured by Sr. Luis Berton, and were presented to the laboratory by Dr. A. M. Walcott.

Female.—Proboscis black with a white ring near the middle; tip somewhat lighter in color, but without a white ring. Palpi dark, with white tips. Mesonotum rubbed; pattern of brassy scales apparently very similar to that in *fasciolata*. Pleurae barred somewhat as in *fasciolata*. Abdomen as in original description. Femora with subapical white ring; lighter at base; very sparsely speckled (especially so on hind femora). Hind tibiae not speckled; mid- and fore-tibiae with extremely few speckles; all tibiae with white ring at outer third, very distinct on hind legs, rather indefinite on front legs. Last three hind tarsals missing; remaining tarsals with narrow basal and apical yellowish-white rings (except last two articulations of fore legs, which are dark as in most specimens of *fasciolata*). Wings show no sub-basal white spot on costa, such as is found in *fasciolata;* small white spot, consisting of 4 to 6 scales, present near base of first vein; not more than four or five white scales scattered on basal half of costa, subcosta, and first vein; none on outer half, none on other veins.

SUMMARY.

Fertile eggs were obtained from *Mansonia* (*Rhynchotaenia*) *justamansonia* and *M.* (*R.*) *fasciolata*. Brief notes are given of the eggs and of first stage larvae. In nature the larvae of these two species, as well as those of *M.* (*R.*) *chrysonotum*, were found attached to the roots of a large sedge, *Fuirena umbellata*. A description is given of the fourth stage larva of *justamansonia*, and a few differences are pointed out between it and the larvae of the other species. Larvae of *M.* (*R.*) *albicosta* were found. A few notes are given of the wing markings and genitalic characters of *fasciolata*. It is probable that *M. albifera* Prado is a synonym of *M. chrysonotum* (Peryassú). A partial description is given of some imperfect specimens of *Mansonia* from Bolivia, which may be *M.* (*R.*) *araozi* Shannon and Del Ponte. Figures are presented of the aquatic stages of three members of this group (principally of *justamansonia*), also of the male terminalia of *fasciolata*.

ACKNOWLEDGMENTS.

The writer takes great pleasure in acknowledging the invaluable help of his assistants, Sr. Nelson Cerqueira, laboratory technician and artist, and Sr. Epiphanio da Silva, field collector.

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EXPLANATION OF PLATES.

Plate I.

Fig. 1. View of swamp, which is known to be a breeding ground for at least three species of *Mansonia*, subgenus *Rhynchotaenia*. Pirajá, near São Salvador, Bahia, Brazil. Fig. 2. Species of large sedge (*Fuirena umbellata* Rottb.) upon whose roots have been found larvae of *M. fasciolata*, *M. justamansonia*, and *M. chrysonotum*.

Plate II.

Fig. 3. Egg raft of *M. justamansonia*, × 10. Fig. 4. Eggs of *M. justamansonia* (semidiagrammatic, in that all air cells are not shown), × 60. Fig. 5. First stage larva, *M. justamansonia*, × 50. Fig. 6. First stage larva, *M. fasciolata*, × 50. Fig. 7. Antenna from first stage larva of *M. justamansonia*, × 250. Fig. 8. Antenna from first stage larva of *M. fasciolata*, × 250.

Plate III.

Fig. 9. Fourth stage larva of *M. justamansonia*, (eighth segment presents lateral surface), × 25. Fig. 10. Comb tooth (second from air-tube), eighth segment, larva of *M. justamansonia*, × 390. Fig. 11. Same from larva of *M. chrysonotum*, × 390.

Plate IV.

Fig. 12. Group of hairs posterior to comb, on lateral surface of eighth segment, fourth stage larva, *M. justamansonia*, × 60. Fig. 13. Same for *M. fasciolata*, × 100. Fig. 14. Same for *M. chrysonotum*, × 100. Fig. 15. Antenna, fourth stage larva, *M. chrysonotum*, × 60. Fig. 16. Antenna, fourth stage larva, *M. justamansonia*, × 60. Fig. 17. Air-tube, fourth stage larva, *M. justamansonia*, × 60. Fig. 18. Saw blade from shoulder of air-tube, *M. justamansonia*, × 390.

Plate V.

Fig. 19. Antenna, fourth stage larva, *M. fasciolata*, × 60. Fig. 20. Mental plate, fourth stage larva, *M. fasciolata*, × 390. Fig. 21. Mental plate, fourth stage larva, *M. justamansonia*, × 390. Fig. 22. Mental plate, fourth stage larva, *M. chrysonotum*, × 390. Fig. 23. Comb tooth (second from air tube), eighth segment, larva of *M. fasciolata*, × 390.

Plate VI.

Figs. 24 and 25. Pupal case, *M. justamansonia*, × 25. Fig. 26. Siphon from pupa of *M. justamansonia*, × 60.

Plate VII.

Fig. 27. Male terminalia, *M. fasciolata*, × 140. Fig. 28. Basal lobe of side-piece, *M. fasciolata*, × 160. Fig. 29. Ninth tergite, *M. fasciolata*, × 160. Fig. 30. Tenth sternite, *M. fasciolata*, × 250. Fig. 31. Mesosome, *M. fasciolata*, × 250. Fig. 32. Mesosome, *M. justamansonia*, × 250.

FIGURE 1

FIGURE 2

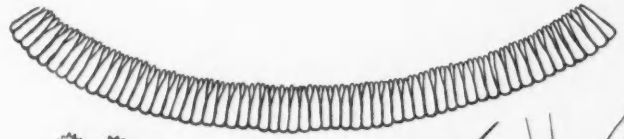


Fig. 3

Fig. 4

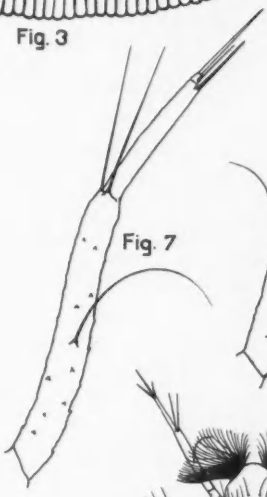


Fig. 7

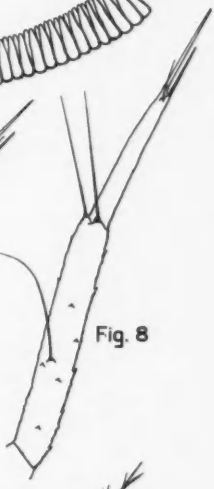


Fig. 8

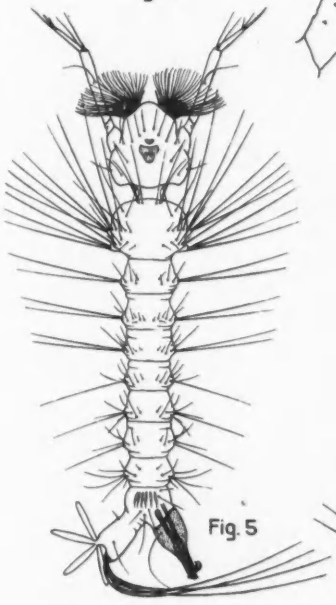


Fig. 5

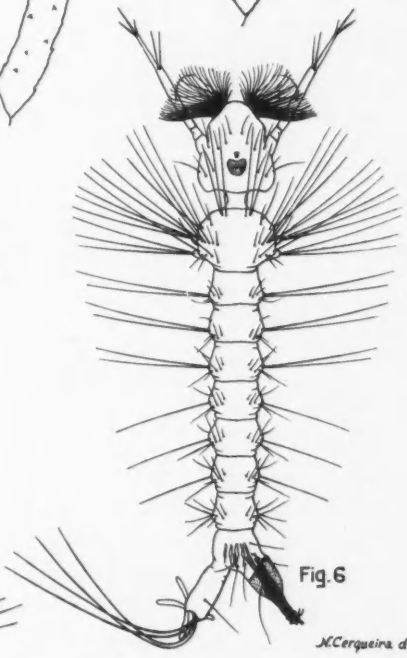
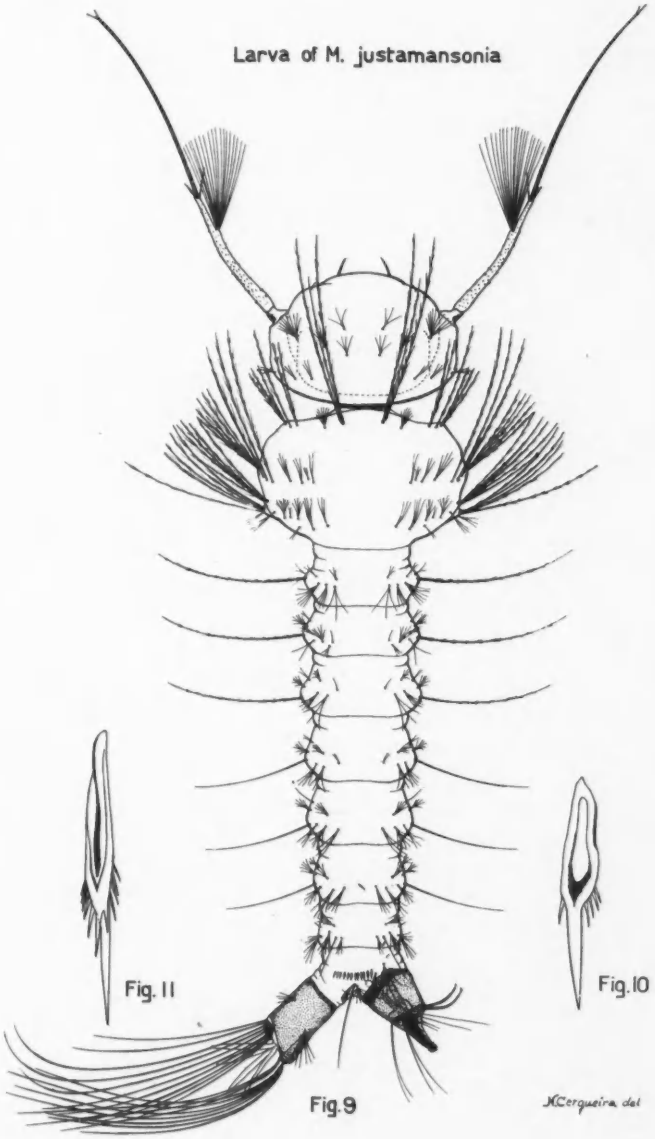


Fig. 6

M.Cerqueira del


Larva of M. justamansonia
Fig. 11
Fig. 10
Fig. 9
M. Cerqueira del

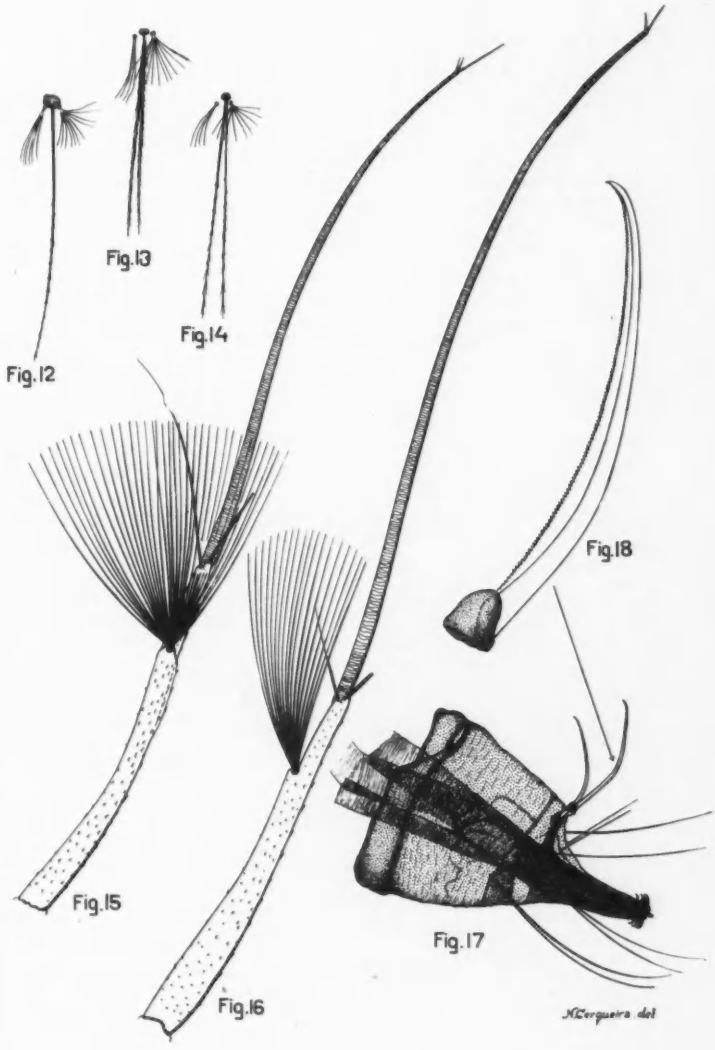


Fig.12
Fig.13
Fig.14
Fig.15
Fig.16
Fig.17
Fig.18
X.Cerqueira del

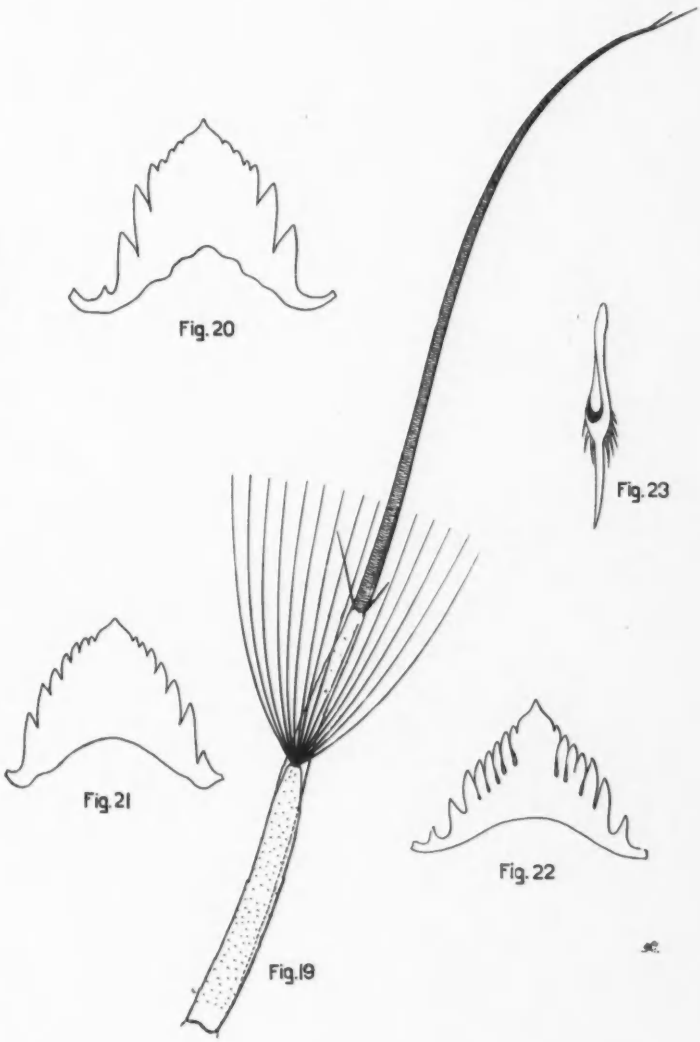


Fig. 20
Fig. 23
Fig. 21
Fig. 22
Fig. 19

Pupa, M. justamansonia

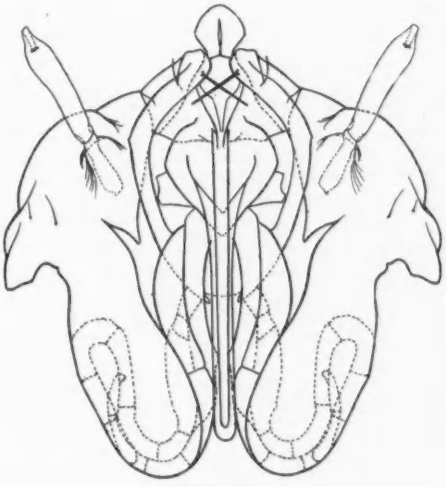


Fig. 24

Fig. 26

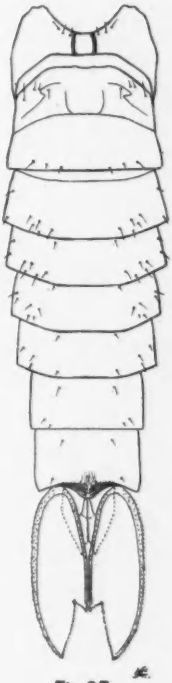


Fig. 25

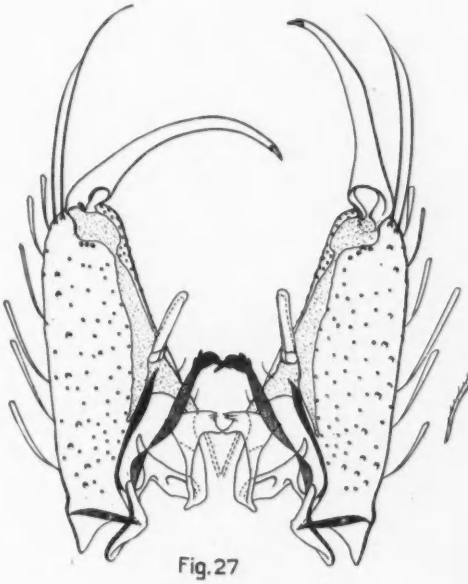


Fig. 27

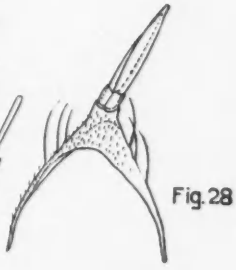


Fig. 28

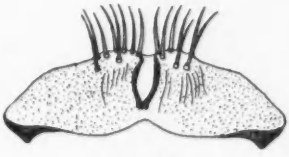


Fig. 29

Fig. 30

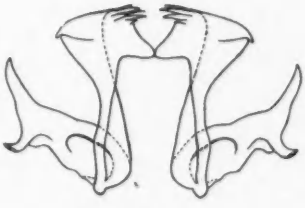


Fig. 31

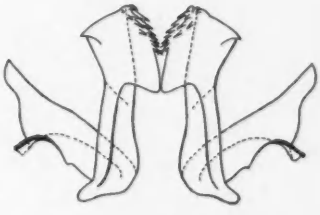


Fig. 32

M. Carqueira del

THE EMBRYOLOGY OF THE BLACK FLY, SIMULIUM PICTIPES HAGEN.

MRS. F. L. GAMBRELL
(Lydia A. Jahn),
Geneva, N. Y.

CONTENTS.

Oviposition Habits of Simulium 642
Methods and Technique 643
Description of Egg 644
Early Developmental Processes Including Maturation, Cleavage, and the Formation of the Blastoderm and Germ Cells 645
Germ Band and Embryonic Membranes 646
Lower Layer and Revolution of Embryo; Segmentation 648
Formation of the Neural Groove, Stomodaeum, Proctodaeum and Appendages. 648
Endoderm 650
Formation of the Oesophageal Valve 651
Mesodermal Derivatives 652
Ectodermal Derivatives 652
Summary 654
Bibliography 655

Insect embryology as a subject of valuable reaserch has extended over a period of years including the latter half of the last and the early part of the present centuries. Many forms have already been described yet each new one reveals similarities and differences that contribute more and more toward a clearer and more comprehensive understanding of the whole field of embryology. Among Diptera, species of *Chironomus* (15), (34), (37), (44); *Simulium* (27), (33); *Tanypus* (15); *Musca* (4), (7), (29), (43), (44); *Cecidomyia* (32), (33); *Miastor* (24b); *Lucilia* (7), (36), (9); *Calliphora* (36), (9); and *Drosophila* (23) have all attracted the attention of embryologists.

While attempting to determine the origin of certain anatomical peculiarities of the insect digestive tract that had been traced into prelarval stages, this study of the embryonic development of the black fly was suggested. The black fly, *Simulium pictipes* Hagen, was chosen because of its abundance in the streams near Ithaca, New York, where this investigation was started in connection with work at Cornell University in the summer of 1931. During the summer months larvae may be seen clinging to the surfaces of flat rocks in shallow, swiftly moving streams, and in the morning hours of bright sunny days the adult females can be detected in their egg laying in

similar locations. This black fly is not of the biting type, a circumstance which added immeasurably to the success of the undertaking.

Thus far no work in which cytological methods were employed has been done on the embryology of *Simulium*. Kölliker (27) recognized its similarity to *Chironomus* and made sketches of whole embryos. Metchnikoff (33) by using the same methods, was able to identify the embryonic membranes and observed the rotation within the egg of three unidentified species. Since that time no other embryological study has appeared. Embryologically speaking *Simulium* agrees with other Dipterous forms and is not outstandingly different from *Chironomus*. The chief differences lie in the fact that the embryonic membranes form more rapidly in *Simulium;* the tail of the latter is immersed more deeply into the yolk; the embryo makes only one rotation; the silk glands are early ectodermal invaginations; and the completed embryo of *Simulium* lies curled in a spiral position within the chorion while that of *Chironomus* is simply coiled in one plane.

Acknowledgments are made to Dr. C. H. Kennedy for helpful criticisms and suggestions and the use of his extensive embryological library; to Dr. O. A. Johannsen at whose suggestion the work was undertaken; to Dr. F. L. Gambrell and Dr. Beulah P. Ennis for their help in collecting; and to Dr. Caswell Grave for use of microtechnical equipment at Washington University, in St. Louis.

OVIPOSITION-HABITS OF SIMULIUM

Black fly eggs were obtained in Fall Creek near the bridge at Forest Home, a small hamlet on the edge of Cornell University Campus. Eggs were found on the upper surfaces of flat stones either at the margin of the stream or at the brink of slight falls which extend from bank to bank at intervals across it. These falls are only from a foot to a foot and a half in height and appear to have been made by faulting and checking in the rock, blocks of which had then disappeared and thus formed a drop in the floor level of the stream. The brinks of these miniature falls had been worn smooth by the constantly flowing water and on such smooth faces the black fly eggs are deposited.

When flies are ovipositing a swarm consisting of perhaps 150 to 200 females collects directly over some smooth flat stone which is thinly covered with swiftly flowing water. They hover about a foot above this area in a restless up and down movement which might be called vertical aerial dance that is maintained over a particular stone for

perhaps twenty minutes or half an hour. Individuals constantly withdraw from the swarm and dip down into the water. During this act some of the eggs which can be seen protruding from their abdomens come in contact with the stone or perhaps leaves, sticks, algae or other matter that might be lying on or attached to the surface. The eggs are completely covered with an exceedingly sticky material much like glue in consistency when the eggs are first laid. This substance has admirable adhesive qualities that can readily be determined by scraping off a mass of newly laid eggs. The material gradually hardens and becomes gritty and brittle.

Egg masses consist of many layers of eggs and are plastered one above another on the rock surface until they sometimes attain a depth of from one-eighth to one-fourth inch. Embryos within any egg mass vary greatly in the stage of their development. In general those on top are farthest advanced, and those that lie on the bottom or toward the center of the egg masses are most retarded unless new masses have been superimposed. The reason for such variations may be found to some extent, in the fluctuation of temperature or oxygen supply or it may possibly be due to the fact that many different females contribute eggs to any given egg mass. It is conceivable that eggs from that many different females may very likely be in varying degrees of development. This variation made it practically impossible to obtain eggs of the same age from any collection, and therefore ages given are only approximate. During the warm months of July and early August the embryonic period was five days in length. Later in August and early September it extended into six or even seven days.

As a general rule the deposition of eggs occurred in the morning, usually between nine and eleven o'clock. However, one night with the aid of a pocket flashlight a swarm of black flies was detected in the act of oviposition at about nine o'clock. How frequently this occurs is not known since it was only observed one time, but it is not unlikely that it may take place repeatedly.

METHODS AND TECHNIQUE.

Collections were taken at half hour intervals except at night when they were taken at two-hour intervals through practically all of the developmental period. For the most part the eggs were killed and fixed and the chorions pricked in the field at an improvised laboratory set up on the bank of the stream. Most of the embryos were killed with hot (70° C.) Kahle's Fluid though Bouin's and Gilson's Fluids were also used. After killing the chorions were pricked to facilitate penetration of the fixative. Pricking the chorions was made less difficult by placing the eggs upon bits of wet blotting paper. Just the right amount of moisture renders the paper of such a consistency that the eggs are supported when the pressure of the needle is applied and thus keeps them from popping out of the field. Needles for puncturing the chorions are made by pushing No. 0 insect pins into the end of a parlor match and grinding the point down sharply upon a bit of limestone. Frequent sharpening is necessary to keep the point in perfect condition.

Fixation varied from six to twenty-four hours, after which the eggs were preserved for varying lengths of time in 70–80 per cent alcohol. After dehydration they were cleared in cedar oil and embedded in paraffin. Since the yolk was not too brittle for sectioning the paraffin method proved satisfactory, nor was it necessary to remove the chorions prior to sectioning. Sections were cut three microns thick and were stained with iron haemotoxylin or Delafield's haemotoxylin with a counterstain of eosin. Various carmine stains were tried with counterstains of picric acid, Orange G and fast green FCF. Best differentiation was brought out with Delafield's haemotoxylin and eosin or iron haemotoxylin. Some effective in toto staining was employed by staining with borax carmine and then destaining with 70 per cent alcohol until the embryo appeared pink and the yolk free of stain.

DESCRIPTION OF THE EGG.

The eggs are elongate, more pointed at the anterior than at the posterior end; and in median longitudinal sections are straight or slightly concave on the ventral side and convex on the dorsal side. They are 0.36 mm. long and 0.2 mm. wide. When first laid the eggs are creamy white in color. This deepens to yellow and then orange during the course of the first day and on the following days the orange deepens into lighter shades of brown, darker shades of brown and finally on the third or fourth day becomes black. The change is due partially to the change in color of the chitinous chorion and partially to the increase in pigmentation of the embryo that lies beneath. The chorion does not become opaque, but remains clear and transparent until hatching.

Two membranes cover the egg, though the cementing substance sometimes clings to the chorion and gives the appearance of a third. The chorion or outer membrane is thin, tough, elastic, structureless and only slightly permeable to fixing fluids. The inner or vitelline membrane is exceedingly thin, structureless and not readily demonstrable. It adheres closely to the egg cytoplasm from which it is usually indistinguishable, and in *Simulium* cannot be detected until late in embryonic life. The micropylar opening, through which the sperm enters must of necessity traverse both membranes, but was not found in any of the sections or whole mounts. From the position of the sperm found in early stages it may lie at the posterior pole. The egg does not completely fill the area within the chorion but shrinks away at the anterior and posterior poles.

Within, the egg is divided into two portions, an inner central meshwork of anastomosing cytoplasm, known as the protoplasmic reticulum in whose meshes the food bodies of different sizes are held; and an outer superficial layer of formative protoplasm that extends continuously over the surface of the egg and in which there is little or no yolk. The yolk is composed of larger or smaller spherical or flattened bodies of a structureless homogeneous character.

Just beneath the surface at the posterior end lies a sharply outlined, irregular, darkly staining body that is completely embedded in the formative protoplasm, Fig. 1, Pl. I. This is the "Keimbahnplasma"

of German authors and has been called the "germ line determinant" by Hegner (16). It may be found in several adjacent sections and appears as an irregular cap over the posterior pole.

EARLY DEVELOPMENTAL PROCESSES, INCLUDING MATURATION, CLEAVAGE, AND THE FORMATION OF THE BLASTODERM AND GERM CELLS.

Maturation.—In some cases the egg nucleus is in the process of polar body formation at the time of deposition and in others early cleavage has already begun. Maturation takes place at the periphery a little back from the anterior pole of the egg. Here the egg nucleus lies within a mass of formative protoplasm; it divides, and forms the first polar body. After the maturation division the female pronucleus may be seen at the periphery near the first polar body, Fig. 1, Pl. I. No other polar bodies or their formation were observed. It is to be regretted that sections in which the sperm was discernible were very scarce, and in fact, only two were found. The earliest position of the sperm within the egg to have been seen was just within the formative protoplasm at the posterior pole where it lay in a coiled position. From there it apparently migrates through the protoplasmic reticulum toward the anterior pole. Fig. 1, Pl. I, shows the sperm, according to the author's interpretation, as a straight rod surrounded by protoplasm, a little more than half way through the yolk, with cytoplasmic rays extending from it out into the yolk. Actual union of the sperm and female pronucleus was not observed.

Cleavage.—Since in some cases segmentation has already begun when the egg is deposited the fertilization nucleus probably begins dividing very soon after fertilization. Cleavage nuclei appear near the center of the egg; are surrounded by an irregular layer of cytoplasm that gives off amoeboid processes into the yolk; but are not enclosed by cell walls. They increase in number by mitotic division, Fig. 4, Plate I, and start migrating toward the periphery soon after their formation. When first formed the nucleus is central in position, but later it attains the peripheral side of the cell.

Blastoderm Formation.—The blastoderm is formed by the cleavage nuclei which migrate to the periphery and come to rest at the surface. Cell walls are not, as yet, present. Arrived at the periphery, they penetrate the layer of formative protoplasm, continue active synchronous cell division until the entire surface of the egg is covered, Figs. 5, 6, 7, 8, 9, 10, 11, Pls. I and II. The axes of the spindles of these divisions are parallel to the surface of the egg, Figs. 8, 10, Pl. II.

Cell walls form first at the outer ends and then at the sides, Figs. 7, 10, Pl. II. The inner ends of the blastoderm cells are in contact with the reticular protoplasm to which they are connected by long filamentous strands for some time after the outer walls and those at the sides have formed. Finally the inner walls have formed and the blastoderm is complete.

Formation of the Germ Cells.—Primitive germ cells, the so-called pole cells are commonly found in Diptera. They represent an early differ-

entiation of the embryonic gonad, and differ markedly from the somatic cells. Their formation and occurrence have been figured and described repeatedly by students of embryology. They were discovered by Robin (39), and redescribed by Weismann (44), Hasper (15), Grimm (11), Balbiani (1), Ritter (37), Metchnikoff (33), and others. One of the early cleavage cells near the posterior pole of the egg moves toward the periphery, passing through the germ line determinant or "Keimbahnplasma" on its way to the posterior end, Fig. 4, Pl. I. The germ line determinant as mentioned before has the capacity for staining deeply, and apparently has some effect upon the germ cells as it passes through. It surrounds these particular cells and enters their cytoplasmic layer thereby lending to them its own peculiar staining properties and making them distinguishable from the remaining cleavage cells. While these cells are undergoing several divisions they migrate toward the posterior pole, and continue dividing until they come to a temporary standstill between the chorion and blastoderm that is now in the process of formation. For a short time they are incorporated among the blastoderm cells but are readily distinguishable by their morphological characteristics and size, Fig. 6, Pl. II. When first seen in the black fly these cells are lying in a group in the posterior portion of the blastoderm. The destiny of these two types of cells (somatic cells and pole cells) is altogether different, the pole cells are the primitive germ cells of the insect whose body will be formed by the remaining cells or the blastoderm. Both types have come from the same fertilization nucleus and have the same inherent characteristics. The germ cells remain at the posterior pole outside of the blastoderm until it is fully formed, Figs. 11, 15, Pls. II, III, and then wander back into the yolk pushing blastoderm cells aside as they go, Fig. 13, Pl. II. About the time that the germ band is forming they have re-entered the yolk, Fig. 14, Pl. III, at the posterior end of the egg; and when it is completed they come to rest on the tail of the developing embryo, where they remain through a series of successive developmental stages. Their development from that point on will be discussed later.

GERM BAND AND EMBRYONIC MEMBRANES.

Germ Band.—Immediately after the return of the germ cells into the egg content a series of changes involving the blastoderm is initiated. The first noticeable modification of the blastoderm affects the length of its cells. At the poles, particularly at the posterior end and along the straight side of the egg, the cells become significantly longer at the expense of the underlying yolk. Coincident with the great increase in length of the blastoderm cells is the flattening of the cells on the curved side of the egg. They are at first round, but as the membranes develop they are reduced to elongate spindle-shaped cells of which the nucleus forms the swollen part. At the anterior pole and along the straight side they are 0.012 mm. in length, at the posterior pole 0.018 mm., and along the curved side only 0.008 mm. in length, Fig. 11, Pl. II.

Shortly after the changes mentioned above, depressions form which increase in depth gradually, one a little back of the anterior pole on the

straight side and another a little before the posterior pole on the curved side, Fig. 15, Pl. III. Coincident with the formation of the depressions other changes are taking place within the blastoderm that finally lead to the formation of the germ band. Along the straight side of the egg and at the poles the cells increase still more in length and undergo marked proliferation, while those on the curved side become flatter, decrease in length and do not proliferate. The first of these changes produces a thickened plate, that extends from anterior to posterior along the straight side of the egg and which is the rudiment of the germ band. The depressions mentioned are the first indication of the embryonic membranes.

Embryonic Membranes.—At the junction of the embryonic rudiment and the flattened blastoderm cells, where the depressions were observed earlier, a double fold begins to form that starts growing over the embryonic rudiment from the sides and along the longitudinal axis, Fig. 15, Pl. III, until the fold from one side meets and unites along the mid-line of the rudiment with the edge of the fold from the opposite side. It starts at the anterior and posterior ends of the germ band and grows over the ventral surface, followed by a growing over from the lateral edges, Metchnikoff (33). The corresponding layers then become continuous and thus two membranes come to lie over the ventral surface of the embryo. The inner membrane is the amnion and the outer membrane is the serosa. The amnion covers only the ventral surface of the embryo while the serosa is a continuous membrane composed of exceedingly flattened cells, that extend in a network over the whole surface of the egg, thus enclosing both embryo and yolk, Fig. 16, Pl. III. The amnion shows active mitotic division during its formation, and is derived from the ectodermal cells at the edges of the germ band where the amniotic fold begins. The serosa, on the other hand, is composed only of the exceedingly flattened cells of the dorsal and lateral blastoderm that have been compressed to the extent that its cells are reduced to thin spindles (in section) with swollen areas indicating the position of the nuclei, Fig. 16, Pl. III.

By the time that both amnion and serosa are completed the germ band has dropped down into the yolk and due to the continued cell proliferation has increased in length anteriorly and posteriorly. Meanwhile the germ cells which have been lying just within the blastoderm at the posterior pole are carried along on the tail of the developing germ band and are immersed in the yolk, Fig. 16, Pl. III. As the embryo continues to lengthen the tail grows around the posterior pole and along the convex side of the egg until it covers about two-thirds of that side, then turns abruptly inward, and curves down into the yolk, Fig. 20, Pl. IV. The germ band is superficial in *Simulium* and extraordinarily long. The curved, hook-like tail as it extends into the yolk may be said to be immersed. In the interim the head has grown around the anterior pole to the curved side of the egg and the entire germ band practically surrounds that region of the egg. The length of the germ band remains constant during gastrulation, segmentation, and embryonic rotation, but then a shortening becomes apparent in the tail region.

LOWER LAYER AND REVOLUTION OF EMBRYO, SEGMENTATION.

Lower Layer.—Gastrulation begins during the formation of the germ band and at about the time of the completion of the embryonic membranes. Before the germ band has lengthened over the curved side of the egg its middle region sinks in along the ventral side forming a furrow along the embryonic rudiment, the gastrular groove, and the sinking portion is in the process of forming the lower layer. The two ridges of this depression form the lips of the blastopore that has extended over the entire ventral surface of the germ band.

Fig. 18, Pl. III, taken in the abdominal region of a day old embryo shows a typical example of the appearance of the gastrular groove. In the middle line there are eight to eleven cells which are sinking inward from the germ band. These will form the future lower layer. Apparently the gastrular groove does not form simultaneously along the entire length of the germ band as sections taken at anterior and posterior ends of the same embryo show no indication of the groove, even though cells all along the mid-line of the germ band are already moving inward, Figs. 17, 19, Pls. III, IV. As the band of cells along the mid-line sinks further into the yolk the lateral parts of the ectoderm close over the vacated area and the result is the two-layered condition characteristic of embryos, Figs. 21, 22, 23, Pls. IV, V. The outer layer is destined to form the body covering, nervous system and parts of the gut, and the lower layer will form mid-intestine, muscle, heart and other tissues, Fig. 24, Pl. V.

Revolution.—Soon after the completion of the germ band the embryo makes a turn of 180° along its longitudinal axis. As a result of this rotation the embryonic parts assume a different relationship than heretofore, in that the germ band now lies in a reversed position along the curved side of the egg, and the yolk lies just beneath the serosa on the straight side, Fig. 32, Pl. VII. Prior to this time the germ band lay along the straight side of the egg.

Segmentation.—Segmentation becomes apparent during the series of events described above. It appears as transverse indentations along the two-layered germ band that are more pronounced at the anterior end and in the lower layer in that the cells are more numerous within a segment than at the boundaries, Fig. 24, Pl. V. The embryo as a whole becomes wider especially at the anterior end where the cephalic lobes are forming.

Approximately the first day of embryonic life ends at this point.

FORMATION OF THE NEURAL GROOVE, STOMODAEUM, PROCTODAEUM AND APPENDAGES.

Neural Groove.—At the end of the first twenty-four hours the embryo has rotated and lies along the curved border of the egg, Fig. 32, Pl. VII. The next significant developmental feature, after the formation of the lower layer and the beginning of segmentation, is the formation of the neural groove. This begins in the middle region of the

body and extends cephalad and dorsad. Primitive neuroblasts appear to either side of the middle of the germ band and lie between the layers of mesoderm and ectoderm, Fig. 25, Pl. V. As the groove deepens the cells of the lower layer along the median line, or "Mittelstrang" of German authors, are released and enter the yolk region. Their destiny from that point is connected with the formation of the mid-intestine and will be discussed later. To either side of the median line and separated by the neural groove lie two lateral longitudinal strips of cells, the mesodermal strips. These strips extend from the cephalic region to the posterior end. Figs. 26, 27, 28, Pl. VI, show the appearance of the neural groove and neuroblasts in different sections of the body at the time of their formation.

Stomodaeum.—Very soon after the formation of the neural groove, a thickening at the anterior end of the germ band becomes apparent. In a longitudinal section this thickening may be seen to consist of an area of elongated cells that is the first trace of the stomodaeum. The elongated cells invaginate, thus forming a depression on the surface of the germ band that appears shortly before the embryo rotates about its longitudinal axis, Fig. 24, Pl. V. It may be seen that the stomodaeum is of ectodermal origin since it is formed from the outer layer of the germ band. At this time there is no indication of a proctodaeum. Prior to the formation of the stomodaeum the mesoderm has extended over the stomodaeal area; as development proceeds and the depression deepens the mesoderm cells are pushed aside and lie near the anterior and posterior borders of the stomodaeum. Figs. 29, 33, Pls. VI, VIII, show a mass of mesoderm cells that lie close to the posterior wall of the stomodaeum and near its inner end so that the two types of cells are practically indistinguishable. Another mass lies anteriorly but not so close as the first mass mentioned. A graphic representation of the progressive development of the stomodaeum may be seen in Figs. 24, 29, 30, 32, 33, Pls. V–VIII.

Proctodaeum.—It has been mentioned above that at the initial appearance of the stomodaeum there is no trace of the proctodaeum. Only after the embryo has rotated and the stomodaeal invagination deepened does the proctodaeal invagination become apparent. This occurs at approximately twenty-four to twenty-eight hours. The germ cells have remained immersed at the tail end of the embryo, Fig. 24, Pl. V, near the amniotic fold. The formation of the proctodaeum, like the stomodaeum, is by means of an ectodermal invagination. It appears near the posterior end of the germ band, just anterior to the amniotic fold, as shown in Fig. 31, Pl. VII. As the depression increases in depth the germ cells are pushed further and further from their former lodging at the extreme end of the germ band to the space between the germ band and the proctodaeal invagination, while the latter sinks more deeply into the yolk. The lumen of the proctodaeum is perpendicular to the amniotic cavity, with which it remains continuous. This manner of the formation of the proctodaeum agrees with that described by Hasper (15) for *Chironomus*. However, according to Weismann's (44) description that is also based on work on *Chironomus*,

the hind-intestine is formed by a splitting in the thickened amniotic fold where it merges with the posterior end of the germ band. The amniotic fold is thus supposed to form one of the walls of the proctodaeum. Ritter (37), also working with *Chironomus*, agrees with Weismann for the most part and there are others, working with *Musca*, who have confused the amniotic cavity with the lumen of the hind gut, i. e., Noack (36), Bütschli (4), Voeltzkow (43), and Graber (10). With the growth of the proctodaeum the germ cells are carried along inward, but have been pushed from the mid-line and separated into two heaps of cells that are figured in a later stage in Fig. 36, Pl. IX.

Near the middle of the second day the Malpighian tubules first become apparent as two diverticula on each side of the tip of the proctodaeum, Fig. 34, Pl. VIII. In this figure the buds are shown in a longitudinal section that is cut near the lateral margin of the proctodaeum. After several hours the tubes increase in length and lie parallel to the body wall. The lumen of the tubules is very small, while the walls are very thick, Fig. 36, Pl. IX.

Segmentation and Appendages.—Meanwhile the body segments have become more pronounced and the first indication of the appendages, especially those of the head, appear at the anterior end, Fig. 33, Pl. VIII. Mesoderm extends down into them. No trace of thoracic or abdominal appendages was observed though a careful study was not made.

ENDODERM.

The ultimate origin of the mid-intestinal epithelium has never been proven to the complete satisfaction of insect embryologists. Although many different conclusions have been reached and many different theories have been advanced none of them satisfy all of the conditions. Among the explanations offered are included the following widely differing suggestions. Dohrn (6) thought that the endoderm was derived from yolk cells; Kowalewsky (29) from the mesoderm; Ganin (8) from proctodaeal and stomodaeal invaginations. Later Kowalewsky (29) described the origin of the mesenteron rudiments as arising from the anterior and posterior parts of the mesoderm which have pulled apart, thus liberating the intervening endoderm cells along the middle strand but leaving some of the endoderm primordium at the two ends. Johannsen (24a) gives a diagrammatic representation of such a condition in Figure 35 of his paper. Lastly Carrière and Burger (5) derive the mesenteron rudiments from the original blastoderm and Hirsehler (22) from the middle strand.

In *Simulium* during the early part of the second day the mesenteron rudiments appear at the posterolateral borders of the stomodaeum as a mass of elongate, rounded cells that are indistinguishable from the mesoderm cells, Fig. 33, Pl. VIII. These cells start growing posteriorly as two lateral strips. At the proctodaeum the mesenteron rudiments lie near the origin of the Malpighian tubules and at the anterolateral angles of the invagination. The endoderm cells start growing forward from these two points. There is no evidence that the endoderm is

formed from any structures other than the mesenteron rudiments. The cells that are released along the middle strand and which are said to be endodermal may contribute to the formation of the mid-intestinal epithelium, but from the author's preparations, a positive statement that such is the case cannot be made.

The first appearance of the mid-intestinal epithelium coincides with the formation of the proctodaeum and the stomodaeum. The cells are elongate and lie along the germ band and parallel to its surface as two lateral strips. Those from the posterior end grow forward and those from the anterior end grow back, and eventually they meet somewhere along the way. The elongate character is maintained in these cells from this time until late in embryonic life when the epithelium assumes the squat, rectangular shape characteristic of the larval epithelium. The splanchnic mesoderm buds off from the mesodermal layer and two longitudinal strips that will later form the muscle layer lie close to the endodermal strips, Fig. 36, Pl. IX.

The two lateral longitudinal strips of mid-intestinal epithelium and splanchnic mesoderm grow dorsally and ventrally as two curved bands of cells, Fig. 45, Pl. XII. The mid-intestine closes first near its attachments to the hind and fore-intestines, and gradually closes from both ends toward the middle due to a rapid proliferation of cells in the endodermal-mesodermal strips. The tube closes ventrally first and later, more slowly, closes dorsally.

FORMATION OF THE OESOPHAGEAL VALVE.

The stomodaeum continues to increase in length as development progresses. During the third day it has become significantly longer, has been surrounded by the mesoderm and the blind end has become thinner, Figs. 37, 40, Pls. IX, X. As the inner end flattens it reflects back upon itself, Fig. 37, Pl. IX. The endoderm is attached to the outer tip of the reflected portion, with mesoderm lying between the latter and the main body of the stomodaeum. In Fig. 36, Pl. IX, a cross-section through the abdominal region, the relation of the splanchnic mesoderm to the epithelium of the mid-intestine becomes apparent. Eventually, with the rupture of the blind end of the stomodaeal invagination the reflected portion increases in length, Fig. 38, Pl. IX, and during the fourth day the contours of the oesophageal valve become distinctly obvious, Fig. 39, Pl. IX, while on the fifth day the completely developed condition of the larval oesophageal valve is obtained, Fig. 43, Pl. XI.

The exact origin of the peritrophic membrane has occasioned much discussion in the literature. Two views concerning its formation are held; one, that the entire mid-intestinal epithelium takes part in its secretion and another, that a group of specialized cells at the anterior end of the mid-intestine secretes the entire membrane. Investigators who have studied the lower Diptera are inclined toward the latter view which is also supported by the embryological evidence from *Simulium*. In the series of sketches, Figs. 37, 38, 39, 43, Pls. IX, XI, the progressive development of the oesophageal valve is portrayed. In the first sketch

the stomodaeal or ectodermal connection of the fore- and mid-intestines is clearly indicated. The outer end of the reflected portion of the stomodaeum forms the place of union with the endoderm and is therefore the area of contention.

In Fig. 38, Pl. IX, the blind end of the stomodaeum has broken through, but the thin, broken, inner wall is still connected to the cells of the reflected portion of the stomodaeum and still remains attached after valve formation has distinctly advanced as seen in Fig. 39, Pl. IX. In longitudinal sections of the larval oesophageal valve the peritrophic membrane takes its origin from a group of cells at the tip of the reflected portion of the stomodaeum in the embryo. In view of this evidence and the author's interpretation of it, the peritrophic membrane arises from a group of cells at the extreme tip of the reflected portion of the stomodaeum which is an ectodermal invagination and therefore the group of cells that secretes the peritrophic membrane is also ectodermal in origin.

MESODERMAL DERIVATIVES.

Before the appearance of the stomodaeum the mesoderm has extended along the longitudinal axis of the embryo in the form of two lateral strips. Near the end of the first day the lateral parts of the mesodermal strips round up into a solid mass of cells that probably represents the coelomic sacs, Fig. 27, Pl. VI, and are segmentally arranged along the germ band, Fig. 24, Pl. V. At no time is there a definite lumen distinguishable and the sacs do not persist after the appearance of the proctodaeum and the stomodaeum. At the cephalic and caudal ends of the embryo the mesoderm forms two distinct cell masses, the anterior and posterior mesenteron rudiments, in which endodermal components are present. There is no way of differentiating between endodermal and mesodermal components of these two cell masses, Fig. 33, Pl. VIII, yet they are unmistakably associated with the formation of the mid-intestinal epithelium.

A layer of splanchnic mesoderm splits off from each of the lateral mesodermal strips and lies next to the mid-intestinal epithelium, Fig. 36, Pl. IX. These cells form the muscles of the intestinal walls, while the muscles of the body wall form from the layer of somatic mesoderm, Fig. 44, Pl. XII.

The mesodermal cells that are released along the middle strand enter the yolk region and there form the blood cells that are found in scattered groups here and there in various parts of the embryo.

During the third day the germ cells lie on a level with the origin of the Malpighian tubules where they remain until hatching. A layer of mesodermal cells surrounds them, Fig. 44, Pl. XII, and they now form the completed embryonic gonad.

ECTODERMAL DERIVATIVES.

The silk glands are invaginations from the ectodermal layer of the germ band and appear toward the middle of the second day after endoderm formation has begun. They lie one on each side of the neural groove in the second maxillary segment. In Fig. 35, Pl. VIII, the

stomodaeum has been cut laterally along its longitudinal axis, which also strikes the silk glands that have invaginated and grown as far as the endodermal layer. Fig. 40, Pl. X, shows a later stage in the development of the glands. They are not shown for their entire length, but are cut off soon after they enter the body cavity. They begin their growth in the second maxillary segment, grow back beneath the suboesophageal ganglion into the body cavity and then grow posteriorly to the fifth abdominal segment where they reflect back upon themselves and grow anteriorly over six segments. In transverse sections through these regions the silk glands are caught four times, and appear as heavy walled tubes with small lumens. Their position is ventral to the mid-intestine in the body cavity, Figs. 36, 45, Pls. IX, XII.

Neuroblasts, referred to as specialized cells that had differentiated shortly before the rotation of the embryo, are found extending all along the segments of the germ band, Fig. 40, Pl. X. From the neuroblasts within each segment are formed the ganglia that lie in a double chain along the ventral side of the embryo, and each of these is connected by nerve strands to the ganglion of the opposite side as well as to those that lie immediately anteriorly and posteriorly. They are thinly covered by a layer of ectoderm externally, Figs. 41, 42, 45, Pls. XI, XII. In the area above the stomodaeum, the large supraoesophageal ganglion, that was formed from the neuroblasts of the first three segments, fills practically the whole space. About the middle of the third day the nerve cord presents thirteen distinct ganglionic masses including the large brain and the suboesophageal ganglion, Fig. 40, Pl. X.

The shortened embryo as seen in Fig. 40, Pl. X, now lengthens out again so that the posterior end grows toward one side of the middle line of the body and the anterior end grows toward the other side with the result that the embryo takes on a spirally coiled position within the chorion. In sections after the middle of the fourth day it is extremely difficult to obtain any accurate picture of development because sometimes three and four portions in as many body regions appear on the same section, due to this spiral coiling of the embryo.

Tracheal invaginations were not found early in their formation but in four and five day embryos tracheal tubes were seen in the abdominal cavity. Trichogen cells are scattered throughout the hypodermal layer of cells.

Early in the fourth day the mid-intestinal wall closes; at that time the dorsal wall has already closed and the heart formed. Early stages in the formation of the heart were not observed, except as heaps of structureless chromatin near the dorsal wall. The cells of the dorsal wall are very thin and elongate when first formed. During the remainder of the fourth and through the fifth day the organs round out their development into the larval form that is present when the insect hatches. Yolk bodies remain visible in the mid-intestine until the chorion ruptures. Some time prior to the latter the somatic muscles become striated and the hypodermis starts chitin secretion. The entire external surface of the embryo is covered with a dense layer of pigmented cells some of which are also found beneath the hypodermis.

The rupture of the amnion and serosa probably occurs just before hatching, though this cannot be said with any degree of certainty since the extremely convoluted condition of the embryo made it difficult to follow. At least they do not rupture before the formation of the body wall and are still seen in five day embryos. At that time they are very thin and have only a few points of chromatin scattered here and there over them.

SUMMARY.

The embryonic period of development in *Simulium* is five days long in mid-summer. Germ cells are a conspicuous feature of the first growth processes. Early in the development they appear among the blastoderm cells at the posterior end of the egg where they lie between the chorion and the blastoderm until the latter is completed and then they migrate inward. Through successive developmental periods they remain on the tail of the growing embryo and are pushed in two heaps on either side of the body by the growth of the proctodaeum that also carries them anteriorly. Eventually as development proceeds they are surrounded by mesoderm and at the time of hatching, when they have formed the completed embryonic gonad, they are located on a level with the origin of the Malpighian tubules.

The embryonic membranes in *Simulium* form more rapidly than in *Chironomus*. In fact the amnion closes on the ventral side before the formation of the gastrular groove or embryonic rotation, both of which take place at the end of the first twenty-four hours. On the dorsal side, the embryo closes toward the end of the third day.

The germ band lies along the straight side of the egg with the head and tail extending well around the curved side. The tail is deeply immersed in the yolk for a part of the time. Soon after the completion of the germ band the embryo makes a 180° rotation upon its longitudinal axis so that it then lies along the curved side of the egg. As hatching nears, the embryo assumes a spirally coiled position within the chorion.

The lower layer is formed by a strip of cells that heave up in the middle of the germ band, instead of being formed by a gastrular tube. The endoderm arises from the mesenteron rudiments that lie in close connection with the stomodaeum and proctodaeum.

The complete development of the oesophageal valve through progressive developmental stages isolates a definite group of

cells, whose origin is ectodermal, at the anterior end of the mid-intestine. These occupy the same area in the embryo as the cells which secrete the peritrophic membrane in the larvae, and are considered to be identical. In the author's opinion this definitely establishes the ectodermal origin of the cells that secrete the peritrophic membrane.

The silk glands are invaginations from the ectodermal layer of the germ band in the second maxillary segment that appear toward the end of the second day.

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EXPLANATION OF PLATES.

PLATE I.

Fig. 1. Longitudinal section of a freshly deposited egg showing polar body, egg nucleus, sperm, and germ line determinant. × 704.
Fig. 2. Division of cleavage cell at periphery showing spindle. × 1,520.
Fig. 3. Longitudinal section of an early stage in the development showing the germ line determinant and four cleavage cells. Composite sketch of a series of sections. × 704.
Fig. 4. Longitudinal section of an egg showing cleavage cells attaining periphery. Composite sketch of a series of sections. × 704.
Fig. 5. Tangential section at the surface of the egg at the time when cleavage cells are migrating to the periphery in blastoderm formation. × 1,520.

PLATE II.

Fig. 6. Longitudinal section taken at the posterior end of the egg during blastoderm formation showing germ cells. × 1,520.
Fig. 7. Cleavage cells at periphery before cell walls have formed. × 1,520.
Fig. 8. Mitotic figures during blastoderm formation showing synchronized cell division. Side walls of blastoderm are forming. × 1,520.
Fig. 9. Blastoderm cells. Side walls forming. × 1,520.
Fig. 10. Mitotic figures during blastoderm formation showing synchronized cell division. × 1,520.
Fig. 11. Longitudinal section of egg after the blastoderm has fully formed. Germ cells lie outside of the blastoderm. × 704.
Fig. 12. Enlarged sketch showing germ cells lying between the chorion and the blastoderm. × 1,520.
Fig. 13. Germ cells passing back through blastoderm. × 1,520.

PLATE III.

Fig. 14. Germ cells have passed back through blastoderm cells and lie within yolk at the posterior pole. × 608.
Fig. 15. Portion of a longitudinal section showing the amnion formation at the anterior end. × 608.
Fig. 16. Longitudinal section through embryonic rudiment after germ band has formed showing position of germ cells, amnion and serosa. × 704.
Fig. 17. Transverse section through anterior end of embryo approximately the same age as embryo in Fig. 16, showing the beginning of gastrulation. Cells in midline elongating and pushing up, though there is no gastrular groove. × 608.
Fig. 18. Transverse section through middle body region of same embryo as 17, showing the beginning of the gastrular groove and the heaving up of the cells in the midline. × 608.

PLATE IV.

Fig. 19. Transverse section through posterior portion of germ band of the same embryo as 17 and 18. Middle cells are elongating, but there is no groove. × 608.
Fig. 20. Longitudinal section through a somewhat later stage showing the elongation of the posterior end of the germ band, the immersion of the germ cells into the yolk, and the hooked tail. × 608.
Fig. 21. Transverse section through embryo about the same stage as 20, showing gastrular groove obliterated and the lower layer. (Through anterior end.) × 608.

PLATE V.

Fig. 22. Transverse section through the same embryo as 21, only in the middle region of the body. × 608.
Fig. 23. Transverse section through same embryonic stage as 21 at the posterior end of the body. × 608.
Fig. 24. Longitudinal section through a 24-hour embryo showing beginning of stomodaeal invagination, segmentation and germ cells. × 608.
Fig. 25. Transverse section through anterior end of embryo in approximately the same stage as 24. Shows beginning of neural groove, and neuroblasts. × 1,520.

PLATE VI.

Fig. 26. Transverse section in head region in same stage as 24, showing neuroblasts and beginning of neural groove. × 1,520.
Fig. 27. Transverse section through abdominal region of same embryonic stage as 24. Shows neural groove, neuroblasts and coelomic sacs. × 1,520.
Fig. 28. Transverse section through same embryonic stage as 24, showing germ cells, neuroblasts, and neural groove. × 1,520.
Fig. 29. Longitudinal section through stomodaeum at about thirty hours, showing cells of lower layer being pushed aside. × 608.

PLATE VII.

Fig. 30. Longitudinal section through stomodaeum several hours later, showing its deepening. × 608.
Fig. 31. Longitudinal section through tail region of a 30-hour embryo showing beginning of proctodaeal invagination. × 608.
Fig. 32. Longitudinal section through both proctodaeum and stomodaeum at about the middle of the second day showing beginning of endoderm formation. × 608.

PLATE VIII.

Fig. 33. Longitudinal section cut laterally through the stomodaeum showing segments and endoderm formation. × 608.
Fig. 34. Longitudinal section through the proctodaeum toward the end of the second day showing Malpighian diverticula. × 1,520.
Fig. 35. Longitudinal section through the stomodeaum at the same age as 34, showing silk gland invagination and growth of the endoderm. × 608.

PLATE IX.

Fig. 36. Transverse section through the posterior end of the germ band of about a 48-hour embryo showing silk glands, endoderm, and splanchnic and somatic mesoderm. × 608.
Fig. 37. Longitudinal section through the stomodaeum showing thinning of the inner wall. × 1,520.
Fig. 38. Longitudinal section through oesophagus showing break of blind inner end. × 1,520.
Fig. 39. Longitudinal section through the oesophageal region of a four-day embryo showing beginning of oesophageal valve. × 1,520.

PLATE X.

Fig. 40. Longitudinal section through embryo, obliquely cut through hind gut, shows shortening and structures at anterior end. × 608.

PLATE XI.

Fig. 41. Transverse section through posterior end showing place where Malpighian tubules come off and relation of germ cells to this region. × 608.

Fig. 42. Transverse section through hind gut in a somewhat later stage showing mesoderm formation about germ cells, and the Malpighian tubules. × 608.

Fig. 43. Longitudinal section through oesophageal valve of a five-day embryo. X 1,520.

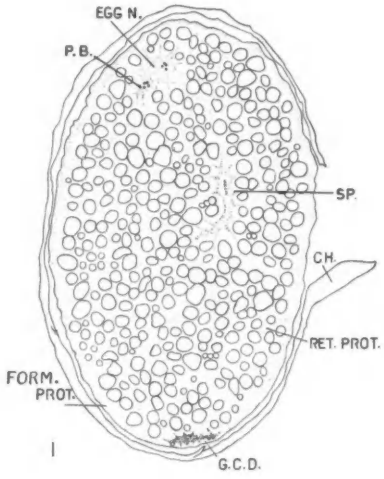
PLATE XII.

Fig. 44. Longitudinal section through a portion of the body wall showing mesodermal layer around germ cells. × 1,520.

Fig. 45. Transverse section through heart. Dorsal and ventral growth of endoderm shown. × 608.

ABBREVIATIONS USED IN PLATES.

Am—amnion.
Am. Cav.—amniotic cavity.
Am. F.—amniotic fold.
Ant.—anterior.
Blast.—Blastoderm.
Blast. C.—blastoderm cell.
B. C.—blood cell.
Body W.—body wall.
Br.—brain.
Ch.—chorion.
Coel.—coelom.
Cl. N.—cleavage nucleus.
Cl. C.—cleavage cell.
D. H.—dorsal heart.
End.—endoderm.
Ect.—ectoderm.
Egg N.—egg nucleus.
Form. Prot.—formative protoplasm.
Gon.—gonad.
G. C. D.—germ cell determinant.
G. C.—germ cells.
H. G.—hind gut.
Labr.—labrum.
Lab.—labium.
Malp. Div.—Malpighian diverticula.
Malp.—Malpighian tubules.
M. G.—mid gut.
Mes.—mesoderm.
Mus.—muscle.
Mand.—mandible.
Neur. G.—neural groove.
N. C.—nerve cord.
Neur.—neuroblasts.
Oes.—oesophagus.
P. C.—pigment cells.
Proct.—proctodaeum.
Post—posterior.
P. B.—polar body.
Ret. Prot.—reticular protoplasm.
Som. Mes.—somatic mesoderm.
Spl. Mes.—splanchnic mesoderm.
Stri. Mus.—striated muscle.
Stom.—stomodaeum.
Seg.—segment.
S. G.—silk gland.
Supraoes. B.—supraoesophageal body.
Suboes. B.—suboesophageal body.
Sp.—sperm.
Ser.—serosa.
Trach.—trachea.
Trich.—trichogen cell.
Y. C.—yolk cell.
Y.—yolk.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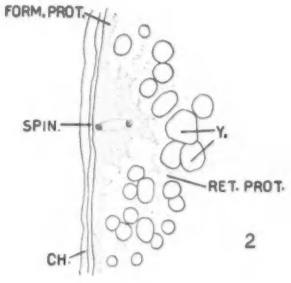
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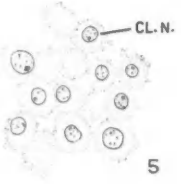
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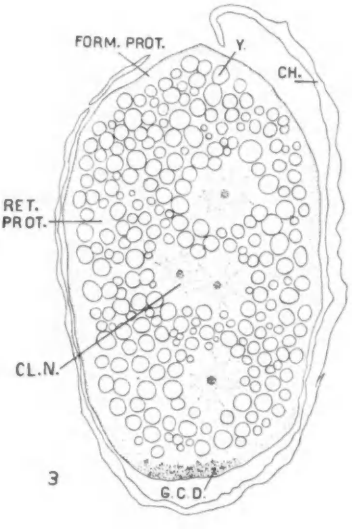
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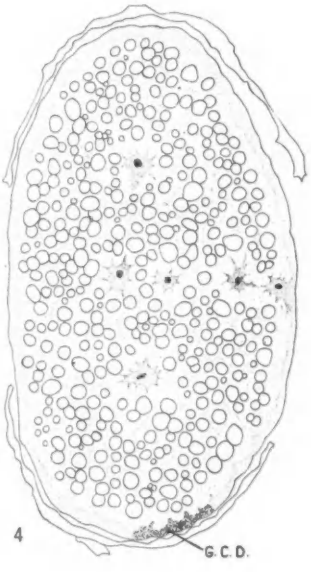
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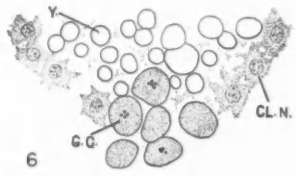

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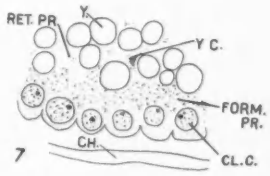

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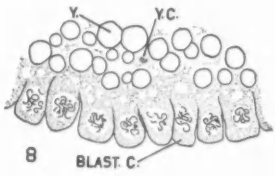

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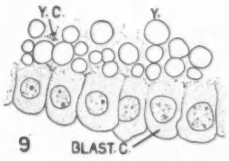

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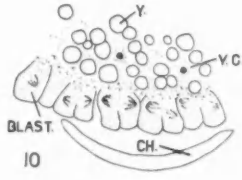

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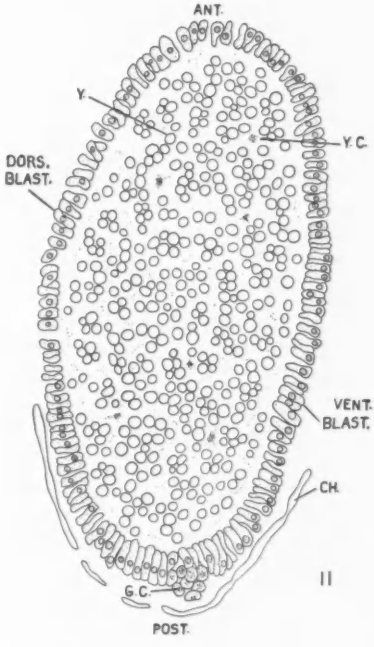

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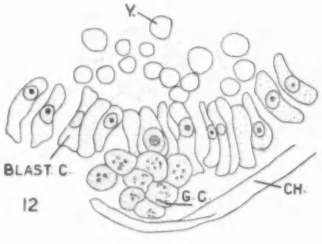

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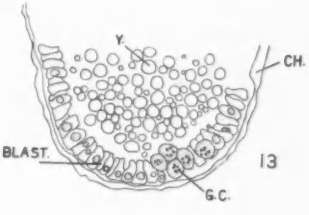

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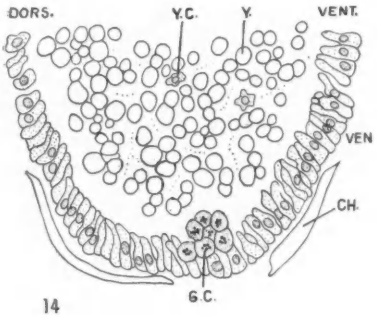
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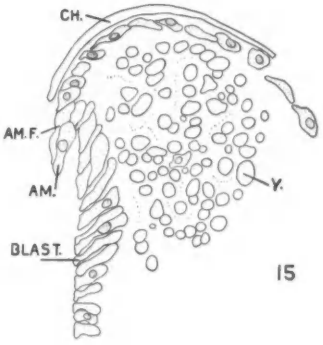
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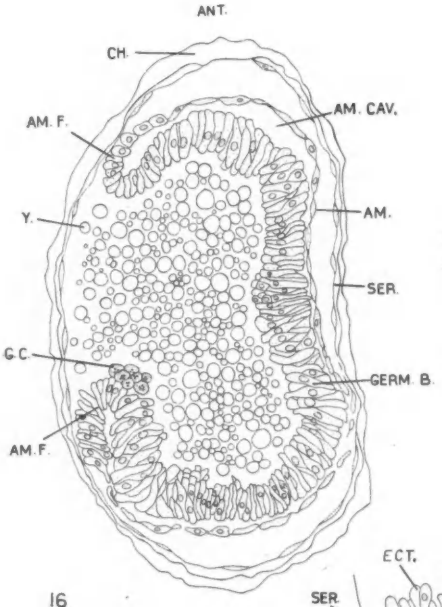
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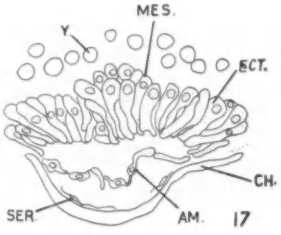
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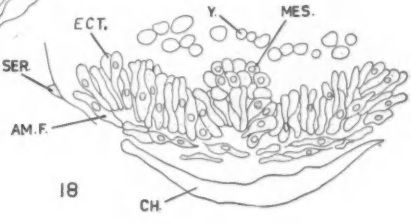
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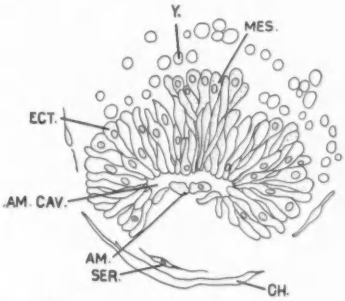
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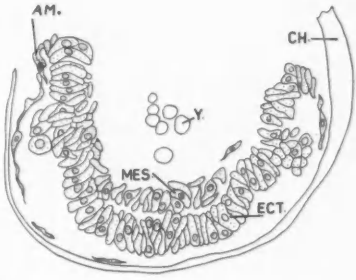
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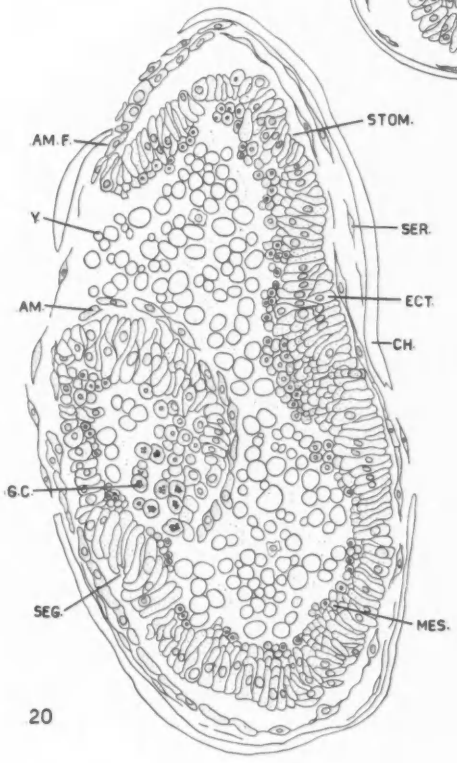
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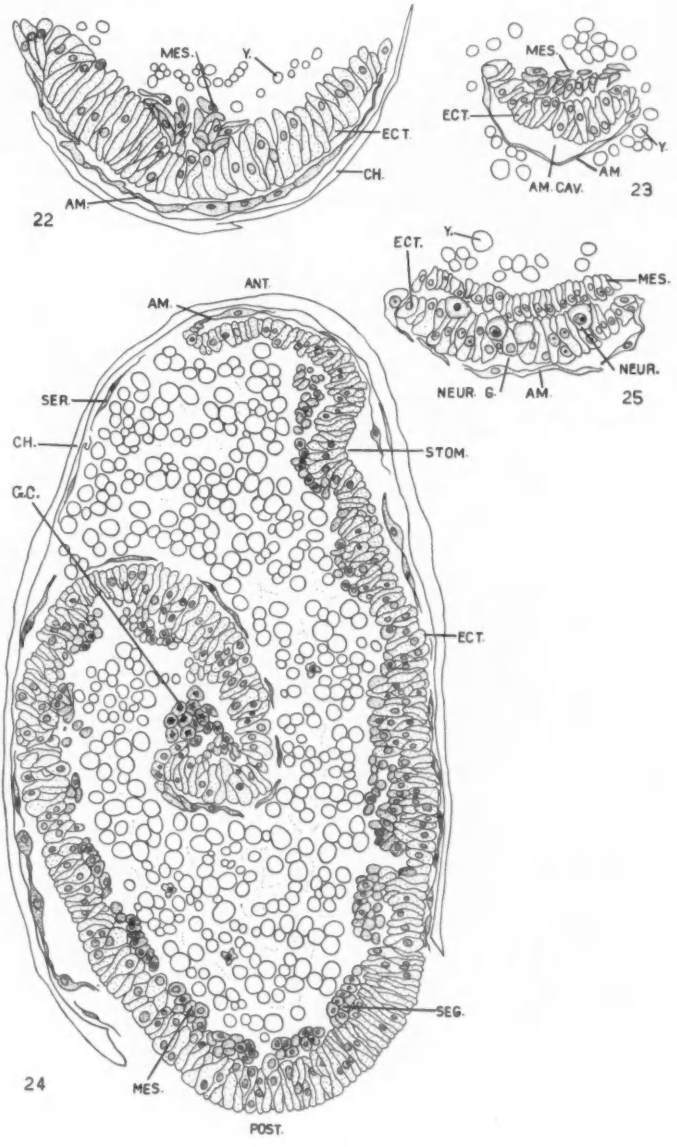
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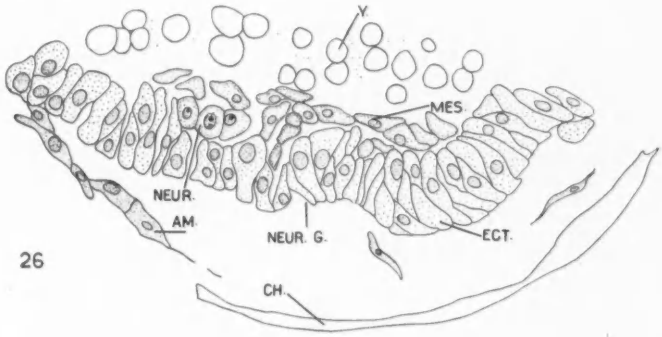
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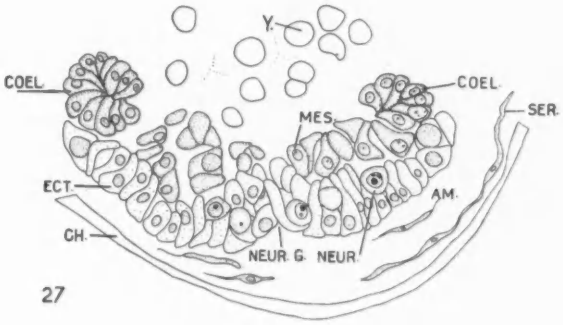
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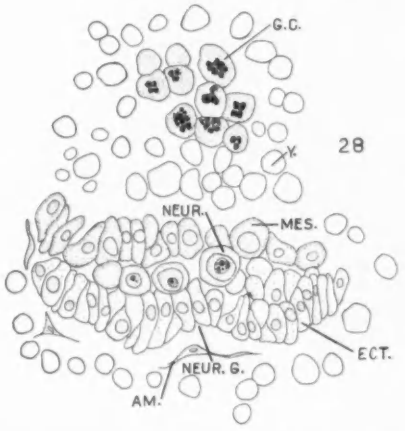
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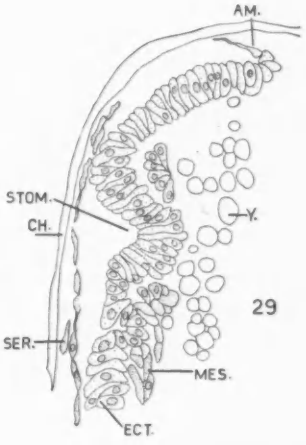
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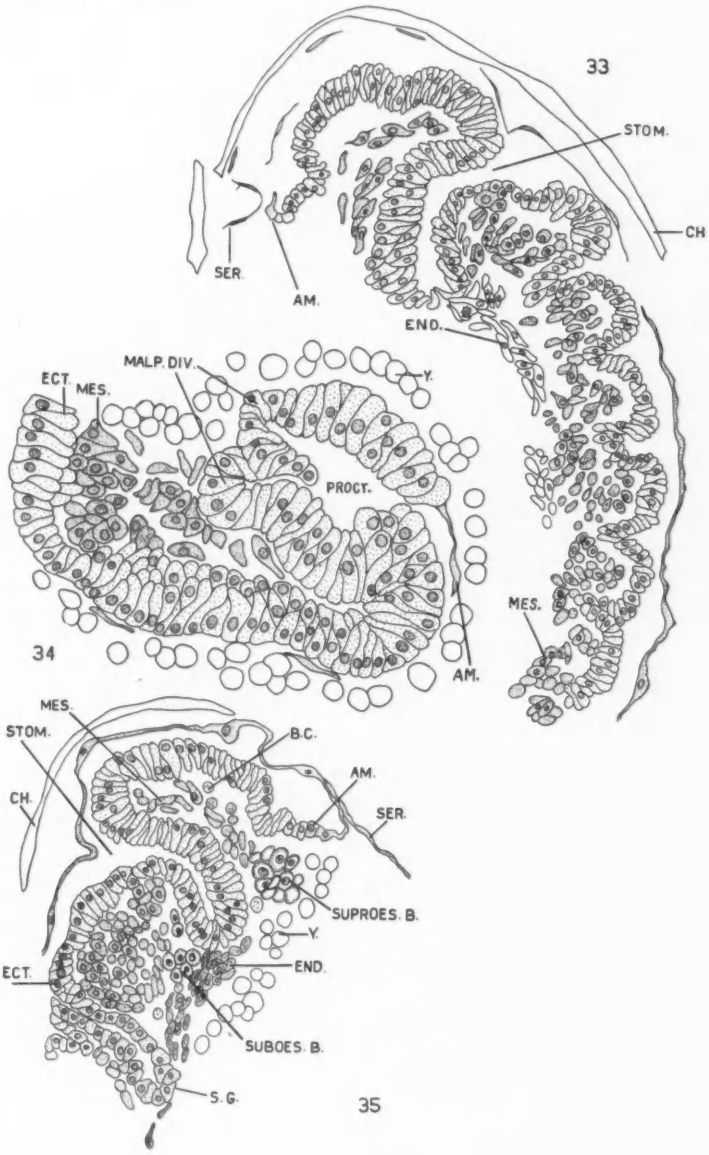
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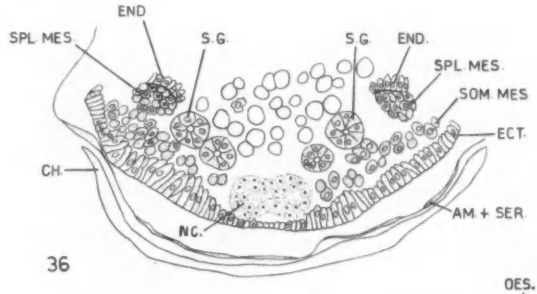
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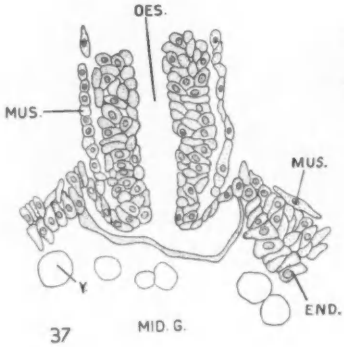
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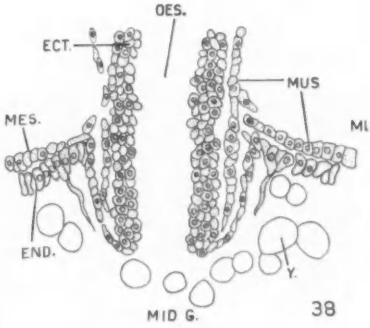
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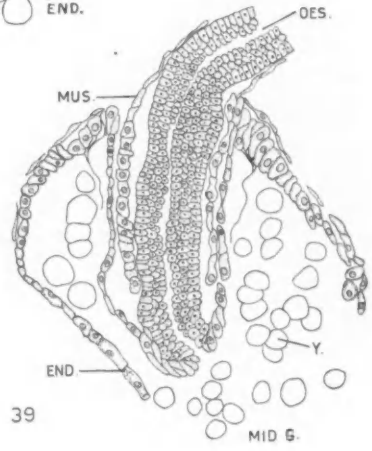
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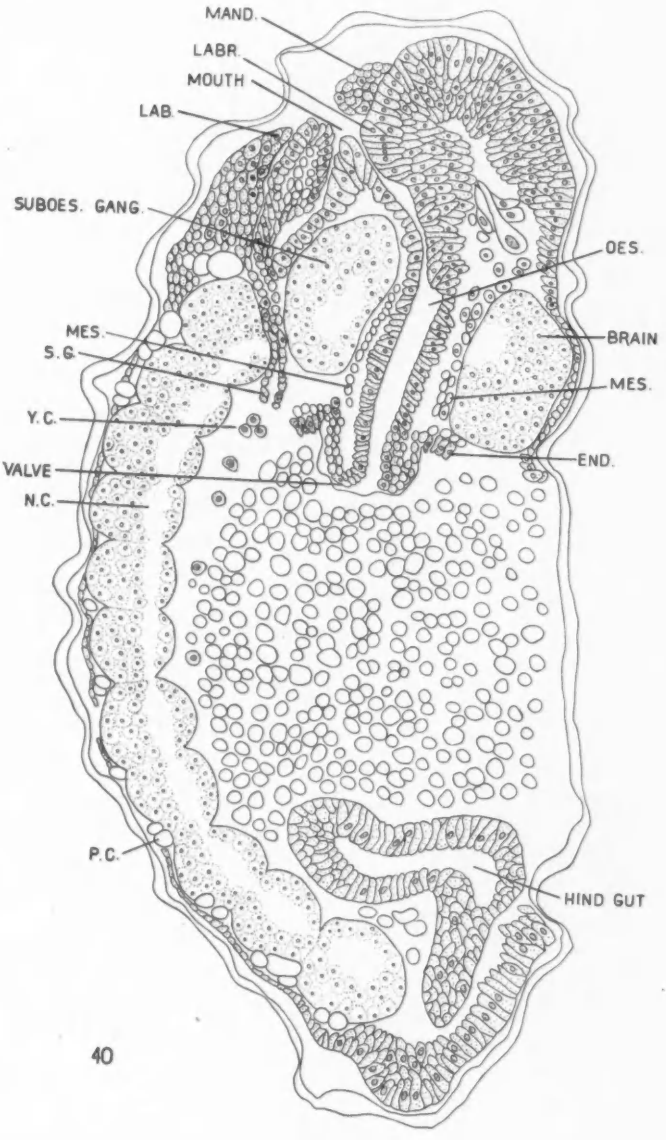
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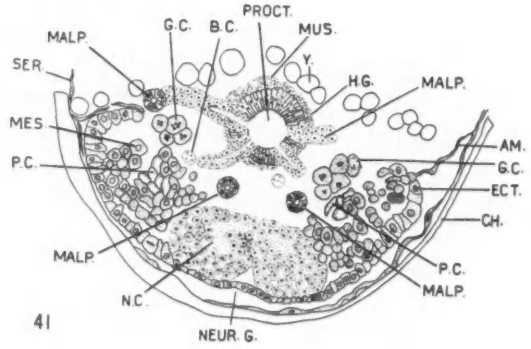
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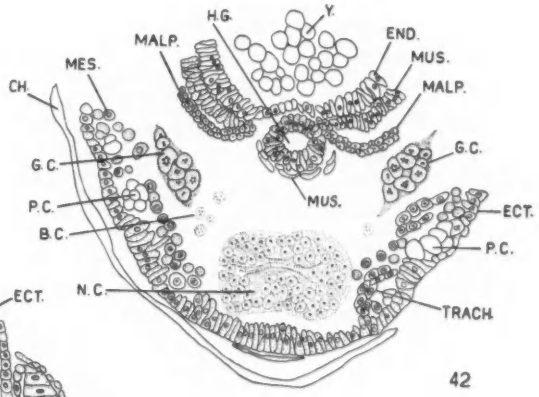
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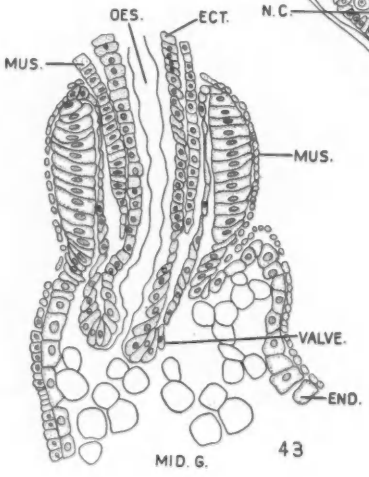
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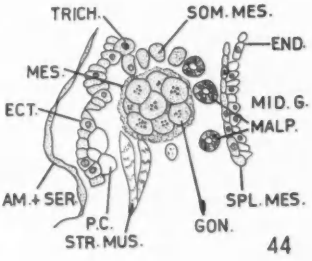
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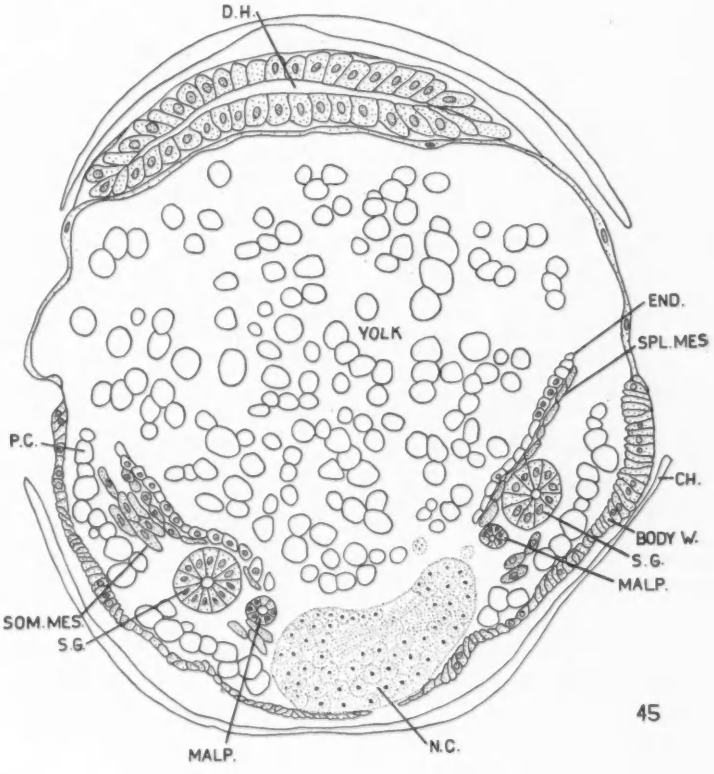
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MALP.
SOM. MES
S.G.
MALP.
N.C.

45

INDEX TO VOLUME XXVI

Acrididae in Colorado, Relations between Altitude and the Distribution of, 586.
Aedes, Notes on, 13.
Aedes oligopistus, 282.
Aedes jacobinae, New Species, 14.
Aedes, Taeniorhynchus, Distribution of, 13.
Aenoplex carpocapsae Cush., Codling Moth Parasite, Variations in the Life Cycle of Male, 345.
Alexander, Gordon, article by, 586.
Amphorophora brevitarsis, 356.
Anatomy of Grylloblatta Campodeiformis Walker. II. Comparisons of Head with those of other Orthopteroid Insects, 309.
Angoumois Grain Moth, Notes on Habrocytus Cerealellae, Parasite of, 536.
Anopheles thomasi, 277.
  maculipes, 277.
  rondoni, 278.
Ants of the Genus Pheidole, Descriptions of two new, 616.
Aphis from Colorado, New Species, 348.
Aphis Gossypii Glover, Color and Color-Variation in, 425.
Application of Artificially Prolonged Hibernation of Parasites to Liberation Technique, 297.
Arbuthnot, K. D., article by, 297.
Atemnidae, 262.
Aulacoyba, 131.
  subitanea, 131.

Baker, W. A., article by, 297.
Baryphyma, 152.
  longitarsum, 153.
Beamer, R. H., article by, 234.
Bean Weevil, Bruchus Obtectus Say, Effects of Ultra-Violet Radiations on the, 603.
Bees Collected by Mrs. Maurice T. James in Pingree Park, Colorado, 40.
Bishop, S. C., article by, 105.
Black Fly, Embryology of, 641.
Blood Coagulation in Several Different Species of Insects, Microscopic Observations, 591.
Book Notices, 94, 198, 199, 261, 276, 302, 308, 376, 380, 423, 424, 559, 562.
Blowfly Larvae in the Treatment of Infected Wounds, Use of, 270.
Bruchus Obtectus Say, Effects of Ultra Violet on, 603.
Butcher, F. Gray, article by, 76.

Capitophorus brevinectarius, 350.
  heterohirsutus, 349.
  longinectarius, 352.
  pullus, 353.
Ceratinops, 109.
  annulipes, 109.
  carolina, 111.
  crenata, 111.
  inflata, 113.
  lata, 114.
  littoralis, 115.
  rugosa, 116.
Chocorua, 164.
  cuneata, 164.
Civilization on the Insect Fauna in Cultivated Areas, Influence of, 510.
Civilization on the Insect Fauna of Forests, Influence of, 497.
Civilization on the Insect Fauna by Purposeful Introductions, Influence of, 518.
Civilization on the Insect Fauna of Regions of Industrial Activity, Influence of, 503.
Claassen, P. W., article by, 503.
Cochlembolus, 167.
  pallidus, 168.
  sacerdotalis, 167.
Cockerell, T. D. A., article by, 40.
Cole, A. C., Jr., article by, 616.
Colletes coloradensis, 41.
  trigonatus, 42.
Colorado Acrididae, 586.
Colorado Aphids, New, 348.
Crosby, C. R., article by, 105.
Chamberlin, Joseph Conrad, article by, 262.
Culex fatigans Wiedemann, Transmission of Yellow Fever Virus by, 491.

Davis, Nelson C., articles by, 13, 277, 491, 619.
Dietrichia, 160.
  hesperia, 160.
Digestive Enzymes of Mosquito Larvae, 45.
Diplotemnus, 264.
  garypoides, 266.
  insolitus, 267.
  insularis, 266.

Dismodicus, 149.
decemoculatus, 149.
Draeculacephala, ovipositor, 64.
Drone Bees, Observations on the Flight and Length of Life of, 239.

Ecological Study of the Ptiliid Myrmecocole, Limulodes paradoxus Matthews, 255.
Ecpantheria (Lepidoptera, Arctiidae), Notes on, 560.
Ellsworth, Joe Koebele, article by, 203.
Embryology of the Black Fly, 641.
Entomological Centenaries, 201.
Enzymes in the Digestive Tract of Mosquito Larvae, 45.
Epameibaphis atricornis, 348.
Eridantes, 146.
erigonoides, 146.
utibilis, 148.
Eulophus viridulus Thoms., A Parasite of Pyrausta nubilalis Hubn., 21.
Euphalepsus liljebladi, 563.

False Scorpions of the Atemnid Subfamily Miratemninae, 262.
Flanders, Stanley E., article by, 529.
Forbes, Wm. T. M., articles by, 490, 560.
Fulton, B. B., articles by, 368, 536.

Gambrell, Mrs. F. L., article by, 641.
Gillette, C. P., article by, 248.
Gould, George E., article by, 465.
Graham, Samuel A., article by, 497.
Grylloblatta, Morphology of, 309.
Grylloblatta Campodeiformis var. Occidentalis Syl, Collecting, 234.
Gyrinidae, Hymenopterous Parasites of, 76.

Habrocytus (Chalcididae), 536.
Halictus jameseae, 41.
croryctes, 40.
Habrocytus cerealellae, Parasite of, 536.
Heat Production and Limitation of Densities in Sitotroga Populations, 529.
Hemiteles cheboyganensis, 77.
cushmani, 76.
Hibernation of Parasites, 297.
Hillemann, Howard H., 575.
Hilton, William A., articles by, 554, 557.
Hinman, E. Harold, article by, 45.
Horcotes, 151.
quadricristatus, 151.
Hormathion, 161.
limnatum, 161.
Hour-glass Spider, Notes on the Life History of the, 568.
Howell, D. E., article by, 239.
Hyblaea, (Lepidoptera, Hyblaeidae), Pupa of, 490.
Hybocoptus, 156.
dentipalpis, 157.
Hymenopterous Parasites of Gyrinidae with Descriptions of New Species of Hamiteles, 76.
Hypomma, 129.
marxii, 130.
Hypselistes, 132.
florens, 133.

Influence of Civilization on the Insect Fauna in Cultivated Areas, 510.
Influence of Civilization on the Insect Fauna of Forests, 497.
Influence of Civilization on the Insect Fauna of North America, 497.
Influence of Civilization on the Insect Fauna of Regions of Industrial Activity, 503.
Influence of Civilization on the Insect Fauna by Purposeful Introductions, 518.
Inheritance of Song in Hybrids of Two Subspecies of Nemobius fasciatus (Orthoptera), 368.

Jahn, Lydia A., article by, 641.

Kakimia cerei, 354.
Kennedy, Clarence Hamilton, article by, 95.
Knight, Harry H., article by, 591.

Larval Head of Parasitic Hymenoptera and Nomenclature of its Parts, 86.
Lawson, Paul B., article by, 568.
Lepidoptera, Hyblaeidae, Pupa of Hyblaea, 490.
Lophomma, 124.
depressum, 125.
pingrensis, 126.
sylvaticum, 127.
umbilicatum, 128.
vaccinii, 128.

McClure, H. Elliott, article by, 345.
MacLeod, G. F., article by, 603.
Macrosiphum atripes, 359.
longirostris, 360.
mertensiae, 361.
tenuitarsis, 363.
Mansonia albicosta, 623.
albifera, 623.
araozi, 624.
fasciolata, 623.
justamansonia, 621.
Mansonia chrysonotum, 280.
justamansonia, 281.
Melissodes fimbriata, 44.

Metcalf, C. L., article by, 1.
Mickel, Clarence E., articles by, 377, 381.
Miller, Forrest W., article by, 473.
Minyriolus, 134.
  aquatilis, 135.
  arenarius, 136.
  castaneus, 138.
Miratemninae, 262.
Morphology, Larval Head, Parasitic Hymenoptera, 86.
Morphology of Larval Bremidae and Key to Certain Species (Hymenoptera), The External, 53.
Morphology of the Ovipositor of Draeculacephala, 64.
Morphology, Nervous System of Larva of Papilio polyxenes, 557.
Morphology of Wooly Aphis, 473.
Mosquitoes, Notes on Some South American, 277, 619.
Mutilla dimidiata Lepeletier, A New Name for, 377.
Mutillidae of Formosa, 381.
Myrmilla hoozana, 383.
Mythoplastoides, 142.
  abruptus, 143.
  erectus, 144.
  exiguus, 145.

Neoconocephalus Lyristes in the Middle West, 303.
Notes on Some South American Mosquitoes, 277, 619.

Odontomutilla taiwaniana, 388.
Origanates, 154.
  rostratus, 154.
Osborn, Herbert, article by, 201.
Ovipositor of Draeculacephala, 64.

Palmer, Miriam A., article by, 348.
Papilio polyxenes Fab., Nervous System, Mature Larva, 575.
Parasites of Gyrinidae, 76.
Parasites, Hibernation, 297.
Parasite of Pyrausta, 21.
Parasite of Habrocytus, 536.
Parasitic Hymenoptera, Head of, 86.
Park, Orlando, articles by, 255, 533.
Parker, H. L., articles by, 21, 217.
Pauropoda from New Mexico, 554.
Pauropus manus, 554.
  nexus, 555.
Pelecopsis, 107.
Pheidole californica var. shoshoni, 618.
  pilifera subsp. artemisia, 616.
Pheidole, Descriptions of Two New Ants of the Genus, 616.
Phlattothrata, 165.
  flagellata, 166.
Photoreceptive Organs of a Flesh Fly Larva, Lucilia sericata, 203.
Pocadicnemis, 139.
  hartlandiana, 139.
  pumila, 140.
Proceedings of the Twenty-seventh Annual Meeting, 183.
Pselaphidae from Costa Rica, A New Species, 563.
Ptiliid Myrmecocole, Limulodes paradoxus Matthews, Ecological Study of the, 255.

Rhagovelia calopa, 466.
  collaris, 466.
  collaris var. pulchra, 466.
  distincta, 466.
  hungerfordi, 467.
  knighti, 468.
  longipes, 469.
  obesa, 469.
  oriander, 469.
  rivale, 469.
  robusta, 469.
  spinigera, 469.
Rhagovelia with Descriptions of a New Species, Some Notes on the Genus, 465.
Ritcher, Paul O., article by, 53.
Robinson, William, article by, 270.

Schramm, Mabel Mary, article by, 95.
Scolopendrella notella, 557.
  numexta, 558.
Serafim, Jose, Jr., article by, 13.
Simanton, W. A., 247.
Simulium pictipes Hagen, Embryology of the Black Fly, 641.
Sitotroga Populations, 529.
Smicromyrme alberici, 421.
  drola, 417.
  drupa, 417.
  guntheri, 417.
  hageni, 417.
  naja, 417.
  norna, 417.
  serpa, 417.
  strandi, 417.
  thalia, 417.
  thia, 420.
  triguttata, 421.
  wotani, 420.
Smith, H. D., articles by, 21, 96, 217.
Smith, Harry S., article by, 518.
Smith, Roger C., article by, 510.
South American Mosquitoes, Notes on, 277.
Sphecodes pulsatillae, 43.
Spiders: Erigoneae, Males, with Cephalic Pits, 105.
Spider, Hour-glass, 568.

Squamulotilla, 383.
carinulifera, 386.
erdae, 386.
formosana, 386.
sauteri, 386.
trituberculata, 386.
tumidula, 384.
Strepsipteron eoxenos laboulbenei Peyerimhoff, Additional Notes on the, 217.
Strumigenys dietrichi, 100.
pergandei, 100.
pulchella, 100.
ohioensis, 98.
Strumigenys with Notes on Ohio Species, A New, (Formicidae: Hymenoptera), 95.
Surface Area of Insects, Determination of the, 247.
Symmigma, 159.
minimum, 159.
Symphyla, Western, 557.

Tapinocyba, 117.
dieterichi, 118.
hortensis, 119.
minuta, 120.
scopulifera, 121.
simplex, 123.
Temnostoma balyras, 4.
bombylans, 4.
Temnostoma Differentiated by its Larval Characters, An Obscure, 1.
Thomas, Edward S., article by, 303.
Timulla (Trogaspidia) orientalis, 377.
Timulla (Trogaspidia), 388.
concava, 402.
debitatum, 400.
discolor, 404.
disparilis, 398.
elpinice, 395.
friekae, 410.
gutrunae, 410.
logei, 413.
minae, 413.
orientalis, taiwanensis, 408.
pfafneri, 413.
philippienensis, amartana, 391.
rapa, 408.
repraesentans, taihorina, 414.
repraesentoides, 411.
rhea, 406.
suspiciosa, 404.
Transmission of Yellow Fever Virus by Culex fatigans Wiedemann, 491.
Tullgrenius, 263.
indicus, 264.

Usinger, R. L., article by, 239.

Vance, A. M., article by, 86.

Walker, E. M., article by, 309.
Wall, Robert E., article by, 425.
Western Symphyla, 557.
Wooly Aphid of the Elm (Schizoneura Americana), Musculature of, 473.

Yeager, J. Franklin, article by, 591.
Yellow Fever, Transmission of by Culex fatigans, 491.

Zornella, 162.
cultrigera, 162.

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